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THE CONFORMATIONS OF ALKYL GLYCOPYRANOSIDES

BY

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A THESIS

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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE CONFORMATIONS OF ALKYL GLYCOPYRANOSIDES submitted by Alfred James Francis Humphries in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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For my mother

Cecilie Eleanor House

ABSTRACT

Through experiments with model compounds, a Karplus-type torsion angle relationship has been developed for nuclear spin coupling between vicinal carbon-13 and hydrogen nuclei linked by σ-bonds through an oxygen to carbon axis. When the relationship was applied to the examination of the conformational properties of the glycosidic bond of the methyl-^{13}C α- and β-D-glucopyranosides, D-mannopyranosides, and 2-deoxy-D-*arabino*-hexopyranosides, the existence of a profound stereochemical influence, of non-steric origin, was discovered. It was found that this influence supplemented, substantially, the steric forces which tend to favour the adoption of one specific rotameric form in both α- and β-anomers. This conclusion was substantiated by interpretation of the $[M]_D$ constants of the methyl glycopyranosides, compared to those of their parent glycopyranoses, and, by the observation of vicinal carbon to carbon nuclear spin coupling between the aglyconic carbon and C-2 of the pyranoid ring. The somewhat larger aglyconic carbon to anomeric hydrogen couplings found in the β-glycopyranosides, compared to those of the α-anomers, were interpreted as arising from slightly smaller torsion angles in the β-series and this interpretation was substantiated by the results of nuclear Overhauser experiments.

When the Karplus-type relationship, mentioned above, was used to investigate the glycosidic-bond rotamers of the ethyl, isopropyl and *t*-butyl D-glucopyranosides, it was found that rotamer preferences similar to those of the methyl glucopyranosides were observed. As the aglycon increased in size, expected increases in the value of $^3J_{C,H}$ were observed in the tetra-*O*-acetyl derivatives in solvent chloroform, but, in the case of the free glucosides in water, no changes were observed in the coupling constant. It was concluded that water increases, in some way, the stability of the preferred glycosidic rotamer. A decrease in the value of $^3J_{C,H}$ was observed in both *t*-butyl β-D-glucopyranoside and its tetra-*O*-acetyl derivative. This decrease was attributed to bond angle deformation of the type termed θ/θ' by Karabatsos.

The extraordinary resistance of the glycosidic bond to rotation, demonstrated here, has important implications for the secondary conformations of natural glycosides and polysaccharides, and provides substantial evidence for the existence of the *exo*-anomeric effect.

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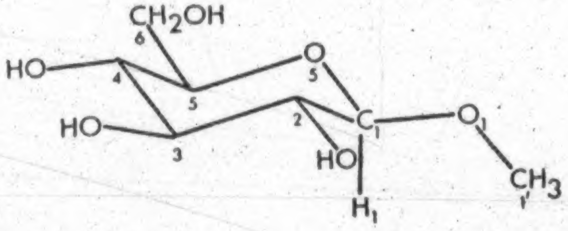
I. INTRODUCTION

A. Objectives of This Study

The profound effects of preferred molecular conformations upon both the physical and chemical properties of organic molecules have been well documented in modern chemical studies (1,2,3). The conformations of carbohydrate molecules are the subject of recent reviews by Angyal (4), and Horton and Durette (5), and of a book by Stoddart (3).

One of the important conformational problems of carbohydrate chemistry has been that of predicting the preferred rotamers about the C-1 to O-1 and O-1 to C-1' bonds of sugars and glycosides. Methyl β-D-glucopyranoside (1) is a compound containing both of these bonds, and the nomenclature to be used in this work may be illustrated by reference to its structure. The C-1 to O-1 bond is commonly termed the glycosidic bond and the C-1 and H-1 atoms



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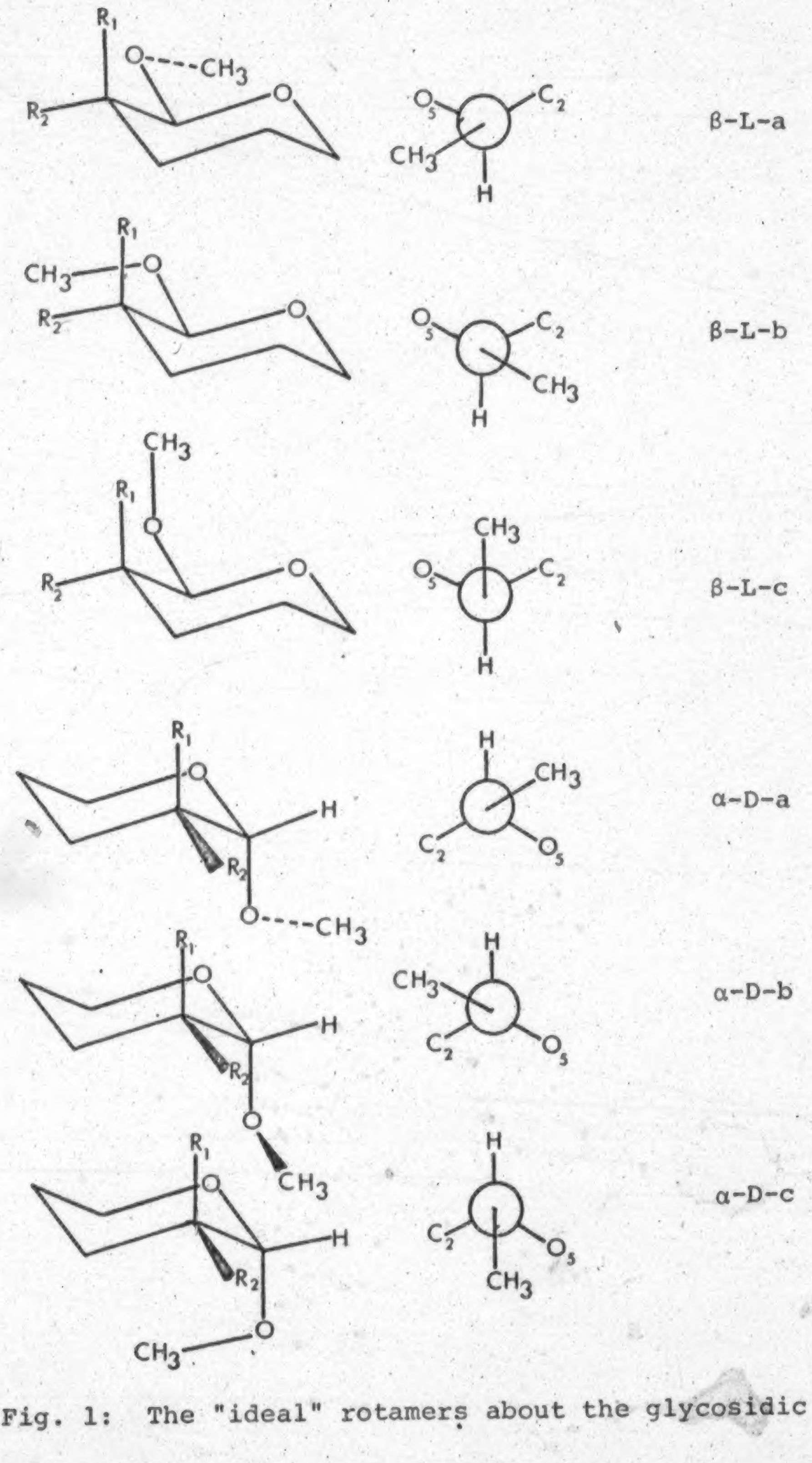

R₁
O
CH₃
R₂
O₅
C₂
H
β-L-a
β-L-b
β-L-c
α-D-a
α-D-b
α-D-c

Fig. 1: The "ideal" rotamers about the glycosidic bond.

are usually referred to as the anomeric carbon and proton (or hydrogen atom), respectively. For the purposes of this work, the O-1 to C-1' bond will be designated as the aglyconic bond and C-1' will be termed the aglyconic carbon. The torsion angle between the aglyconic carbon and the anomeric proton will be designated by the symbol ϕ, while the torsion angle between the anomeric carbon and a specific substituent upon the aglyconic carbon will be designated by ψ. Because only pyranosides have been investigated in this work, the term glycoside, when used here, is intended to refer to pyranoid glycosides, exclusively.

Glycosidic structures are of major importance to medicine and industry being found, for example, in aminoglycoside-type antibiotics, cardiac glycosides, the nucleic acids, gum arabic, starch and cellulose. The properties of these compounds can be expected to be considerably dependent upon conformational details such as rotamer populations about the glycosidic bond. The idealized, staggered rotamers of these bonds are illustrated in Fig. 1.

This thesis is primarily concerned with the possible use of carbon-13 to vicinal proton nuclear spin coupling in establishing conformational preferences. To this end, the carbon-13 enriched methyl glycosides of D-glucose (R_1=H, R_2=OH, in Fig. 1), D-mannose (R_1=OH, R_2=H, in Fig. 1) and 2-deoxy-D-*arabino*-hexopyranose (R_1=R_2=H in Fig. 1) were synthesized, and the vicinal nuclear spin coupling constants between the aglyconic carbon and both H-1 and C-2 of the pyranosyl moiety were determined in several solvents. The results obtained, together with those from nuclear Overhauser double resonance technique experiments with non-enriched methyl glycosides, will be presented and discussed.

The D-glucosides of ethanol-1-^{13}C, isopropanol-2-^{13}C and *t*-butanol-2-^{13}C were also synthesized, and the vicinal carbon-carbon and carbon-proton coupling constants were determined from their n.m.r. spectra, in order to examine the effect of increasing substitution at the aglyconic carbon.

The data obtained will be discussed in terms of steric and anomeric effects and their role in determining a rotameric preference about the C-1 to O-1 bond, and, thus, the secondary conformation of glycosides.

While steric effects are well understood (2), there are many different views of the nature of the anomeric effect and these will now be discussed together with the systems in which it has been observed or inferred.

B. The Anomeric Effects

1. Conformational Definition

In carbohydrate chemistry, the anomeric effect refers to the tendency in pyranoid sugars for electronegative substituent atoms, when bonding to C-1, to adopt *syn*-clinal orientation with respect to the C-5 to O-5 bond; that is, to have axial disposition on the pyranoid ring. Typical examples may be found with oxygen, or halogen atoms as the electronegative substituent (3).

The observed relatively greater thermodynamic and chemical stability of α-glycopyranosides, as compared to the β-anomers, is evidence for the existence of this effect (6). Since there is a well-demonstrated (2) preference for substituents on six-membered rings to adopt equatorial dispositions, it is seen that the anomeric effect acts in a manner opposed to intuitive reasoning based upon the "steric size" of substituents. In glycopyranosyl halides, the preference for axial orientation is so great that it may cause ring inversion (7,8). Furthermore, the relative instability of β-glycosyl halides has been utilized in the development of a recent, stereoselective synthesis of α-glycopyranosides. In this synthesis, excess halide ion is used to cause the formation of a small, but highly reactive, quantity of β-glycosyl halide. This species then reacts preferentially with alcohols, by displacement, to give several otherwise difficult-to-synthesize α-glycosides (9).

Numerous non-carbohydrate compounds have also been shown to have conformations determined by the anomeric effect. For example, 2-chloro- and 2-bromo-tetrahydropyran, *trans*-2,3-dihalogeno-tetrahydropyrans, and *trans*-2,3- and 2,5-dihalogeno-1,4-dioxanes all adopt conformations in which the halogens are axially disposed (10,11,12).

An extension of the anomeric effect was proposed in 1964 (13), and the name *exo*-anomeric effect has recently been suggested for it (14). This extension may be defined as the tendency for the aglyconic bond to exist in *syn*-clinal orientation with respect to the C-1 to O-5 bond to adopt the β-a, β-c, and α-a rotamers, illustrated in Fig. 1. A simple model compound, in which an *exo*-anomeric effect is possible, is methylal (dimethoxymethane). The three "normal" rotameric structures possible for methylal (2), are shown in Fig. 2.

Using principles commonly accepted and applied (2) in the analysis of aliphatic compounds, the most favoured rotamer would be predicted to be 2a since it avoids *syn*-clinal interactions between the relatively large oxygen atoms and the methyl groups. Similarly, the next most favoured would be 2b and the least favoured, therefore, would be predicted to be 2c However, if the *exo*-anomeric effect is considered, the predicted order of rotamer stability would be exactly the reverse, that is, 2c > 2b > 2a. Evidence for the correctness of this latter prediction will now be presented.

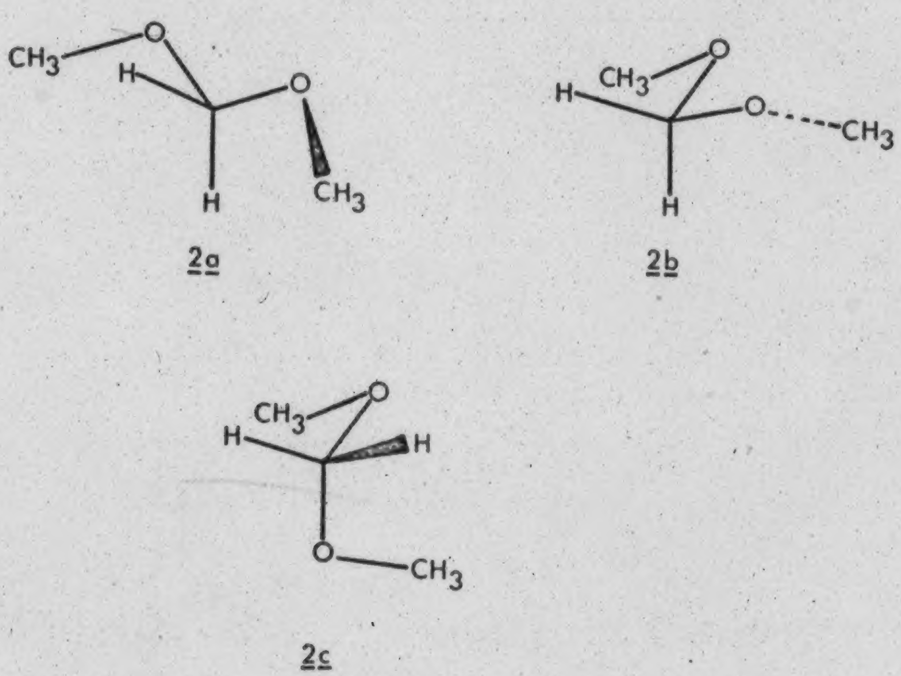


Fig. 2: The conformational rotamers of methylal (2).

2. Evidence of The Existence of The Anomeric Effects

For the case of methylal, discussed above, the most preferred rotamer has been found to be 2c, both by electric dipole moment and electron diffraction studies (15,16). Similarly, chloromethyl methyl ether has also been found (17) to adopt the *syn*-clinal rotameric orientation, predominantly, as do the hydroxyl bonds of chloral hydrate, with respect to the C-O bonds vicinal to them (18). The polymeric analogue of methylal, polyoxymethylene, has been found to have an all *syn*-clinal helical conformation (19), in contrast to polymethylene, which adopts a conformation similar to 2a of Fig. 2. This fact has, of course, important implications for the secondary conformations to be expected in oligo-, and polysaccharides.

Conformations 2b, and 2c, of Fig. 2 are analogous to the β-a and α-a rotameric forms, respectively, of glycopyranosides, and there is evidence that, in both crystalline and solvated states, these are the predominant rotamers for these compounds. Havinga and coworkers have interpreted their electric dipole moment and nuclear spin coupling constant data, obtained for six 2-alkoxytetrahydropyrans, as indicating the existence of only the α-a conformer in the axial anomers, and either the β-a, or β-c conformers, for the equatorial anomers (20).

In the crystalline state, X-ray data for all cases reported so far, confirm that only the α-a and β-a (Fig. 1) rotameric forms are found. Recent high resolution X-ray diffraction studies have demonstrated small, but reproducible, bond length variations, which tend to confirm certain proposals concerning the physical origin of the anomeric effect (21).

3. Physical Origin and Quantum Mechanical Calculations

The physical origin of the anomeric effect has been much discussed, and a number of explanations have been put forward.

In his original report, Edward (6) rationalized the greater stability of α-glycosides as arising from electrostatic repulsive interactions between the resultant dipole, due to the presence of unshared electron pairs on the ring oxygen and the glycosidic bond oxygen atom and that of the C-1 to OR bond, as shown in Fig. 3.

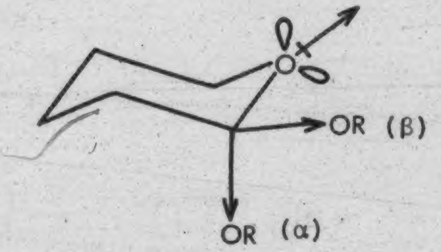


Fig. 3: Dipolar interactions of the glycosidic bond (6).

The polar nature of the effect was, thus, assumed from the beginning, and, for example, Eliel (22) has reported results on the chemical equilibration of 2-alkoxytetrahydropyrans which support this assumption. The free energy associated with the acid-catalysed equilibration of 2-methoxy-6-methyltetrahydropyran was found to vary from

0.74 kcal/mole in solvent carbon tetrachloride to 0.35 kcal/mole in acetonitrile in favour of the axial anomer. It thus appears that the equatorial anomer is significantly less favourable in solvents of low dielectric constant, suggesting that the equatorial form is more polar than the axial. A rough correlation of the magnitude of the anomeric effect with solvent dielectric constant is possible, but deviations occur, apparently as a result of specific solvent effects (23).

Lemieux and Chü suggested that electrostatic, non-bonded interactions between the C-5 to O-5, and C-1 to O-1, bonds were minimized in the α-glycosides (24). It is noteworthy that a similar argument has been advanced to explain the relatively lower-field resonance of equatorial, as compared to axial protons in cyclohexanes (25).

Eliel has pointed out that, in all cases, the anomerically disfavoured rotamer is that in which the greatest number of *syn*-axial lone pair-lone pair repulsive interactions can occur, as viewed by the "rabbit-ear" atomic orbital representation for the spatial location of lone electron pairs (26).

Romers, Altona, Buys and Havinga (12) have proposed a fourth general rationalization for the anomeric effect, based upon their observations in halogeno-1,4-dioxanes, -thioxanes, and -dithianes and in chloromethyl methyl ether, that one of the bonds involved in the anomeric-type linkage systems was shorter than usual, while the other was longer. In chloromethyl methyl ether, the C to Cl bond was lengthened, while the C to O bond was shortened, in comparison to alkyl-halide and ether bond lengths. In the chlorodioxanes, the axial C to Cl bond was 1.819Å, while the equatorial C to Cl bond was 1.781Å long. They concluded that these "abnormal" bond lengths "suggest a description, in which nonbonding electrons on oxygen are delocalized by quantum mechanical mixing of the *p*-orbital of oxygen, with the suitably-oriented antibonding σ-orbital, of the C to halogen grouping." This type of delocalization would effectively strengthen, and thus shorten, the C to O bond, while weakening, and thus lengthening, the carbon to halogen bond, as shown in Fig. 4.

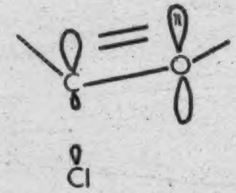


Fig. 4: Orbital mixing and delocalization in chloromethyl methyl ether. [The oxygen atom is sp^2-hybridized due to mixing of excited states. The carbon and chlorine antibonding orbitals only are shown for clarity (12)].

When the C-O bond lengths were compared in a series of halogeno-1,4-dioxanes, -thioxanes, and -dithianes, the C-2 to X bond, as defined in Fig. 5, was found in all cases (except for *trans*-2,5-dibromo-1,4-dithiane where the measurements were not sufficiently accurate), to be significantly shorter than the C-6 to X bond. The C-6 to X bonds are of "normal" length, while the C-2 to X bonds are shorter than usual. These observations are consistent with the proposed orbital mixing mechanism for the anomeric effect.

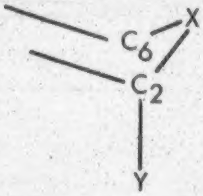


Fig. 5: The C-X-C-Y fragment in halogeno-1,4-dioxanes, -thioxanes, and -dithianes, [X=O or S,Y=Cl or Br (12)].

The results of two different quantum mechanical investigations, which have recently been reported, give partial support to two of the above explanations.

Wolfe and coworkers have carried out *ab initio* quantum mechanical calculations for the model compound fluoromethanol, using the Hartree-Fock approach, and based upon an analysis of attractive-dominant, versus repulsive-dominant interactions (27). Four factors were viewed as contributing interactions:

(a) nuclear-nuclear repulsion,
(b) electron-electron repulsion,
(c) nuclear-electron attraction, and
(d) kinetic energy.

The authors calculated the internal torsion angle dependence of the energy contribution from each factor, and of the total system energy, and were able to duplicate the experimentally observed *exo*-anomeric behaviour of their model. Since the form of the electron-electron Coulombic potential did not differ from other repulsive potentials, the authors conclude that dipole-dipole repulsion between localized lone electron pairs, was not the controlling factor in the anomeric effect, as had been suggested (26).

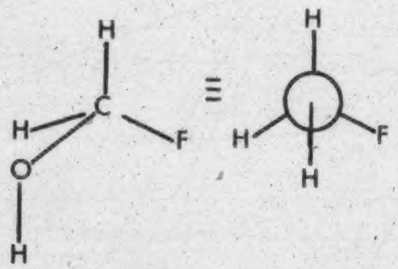


Fig. 6: The preferred rotamer of fluoromethanol (27).

These authors also found, that by averaging the total energy angular dependence curves for hydroxylamine, and hydrogen peroxide, they obtained a curve which corresponded to that of methanol within reasonable limits. From this, the authors conclude, that the origin of the barrier represented by the anomeric effect, "can be understood, principally, in terms of interactions of the bonded electron pairs."

Finally, the observed constancy of the torsional angle dependence of the total energy, kinetic energy, and potential energy curves, in the region between 120° and 240° of torsional rotation, is indicative that the lone pair electrons do not behave as directed "rabbit ears." In this arc, "rabbit ear" electron pairs would eclipse with the carbon to fluorine bond twice, however, no energy changes are observed which would reflect such eclipsing interactions. The authors conclude therefore that their calculations are completely in accord with the earlier rationalization of Lemieux and Chü (24).

A series of molecular orbital theory investigations by Pople (28,29) and a recent paper by Jeffrey, Pople and Radom (30) have served to unite quantum mechanical theory and the experimentally observed facts for many carbohydrates, and to provide theoretical support for the quantum mechanical mixing mechanism proposed by Havinga (12). Calculations were first carried out as part of a general investigation of simple compounds of the first row elements, and of the variation of their system energies, conformations and bond interactions with internal rotation (30,31). When the calculations were performed for the model compound dihydroxymethane, a deep potential energy well was found for the conformation in which the hydroxyl bonds were oriented *syn*-clinally with respect to the C-O bonds in vicinal relation to them. The calculations were further analysed by factoring the total internal-rotation-dependent energy equation into its one-, two-, and three-fold Fourier components. In this way, the variations in energy could be determined and evaluated as a function of internal rotation. These calculations also duplicated the *exo*-anomeric effect, since the controlling preference was found in the one-fold component. Conformational control by this component indicates a profound preference for the *syn*-clinal arcs, and a strong destabilization of the *anti*-periplanar arc. This was attributed by these authors to unfavourable dipole-dipole interactions, of the type first proposed by Edward (6).

Some evidence in support of the theory of Havinga was provided by the observation that the two-fold Fourier component analysis indicated a preference for the orthogonal, as opposed to either planar configuration, when one of the torsional rotors was held at 180° while the other was permitted to rotate. The orthogonal torsional position has the most favourable orientation for orbital-mixing charge-delocalization. In this orientation, electron withdrawal into one carbon to oxygen bond induces partial emptying of the 2p-orbital of the carbon atom, and facilitates the delocalization of the 2p-type, lone pair electrons of the other oxygen atom. This arrangement is illustrated in Fig. 7. If the preference for orthogonal orientation persists in the glycosides, then it would be expected to result in an increase in the torsional separation of O-5 and the aglyconic carbon in the β-anomers, which have an analogous system orientation, with one rotor fixed at 180°, and one nominally free.

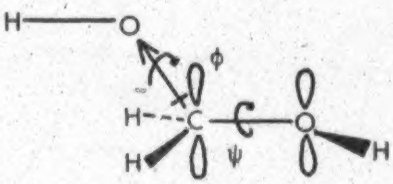


Fig. 7: Stereochemical representation of charge delocalization in methanediol (30). $\phi = 180°$, $\psi = 90°$.

In summary, both dipole-dipole and delocalization effects are predicted to be important, as is demonstrated by the higher energy found for the +*syn*-clinal, -*syn*-clinal, form compared to the +*syn*-clinal, +*syn*-clinal form. The only important difference between the two forms is the existence of unfavourable dipole-dipole interactions.

Variational calculations were also carried out in which the various bond lengths were altered until the total system energy was at a minimum. These calculations predict a relative bond shortening for both the +*syn*-clinal, +*syn*-clinal, and the *anti*-periplanar, +*syn*-clinal rotamers, when compared to bond lengths in methanol. Moreover, the latter rotamer was calculated to have unequal bond lengths, and this is consistently observed in the conformationally similar β-glycosides, which have the exocyclic anomeric bond significantly shorter than the endocyclic bond (30).

Based upon these calculations, it can be predicted that the *exo*-anomeric effect will exert a controlling tendency, in both α- and β-glycopyranosides, for the aglycon to adopt -*syn*-clinal orientation in the case of the β-D-glycosides, and +*syn*-clinal orientation in the case of the α-D-glycosides, with respect to the C-1 to O-5 bond. Furthermore, it can be predicted that the torsion angle with respect to the ring oxygen will be slightly greater, and the anomeric bond length slightly shorter in β-glycosides, as compared to α-glycosides. These predictions are indicated in the crystalline state (31,32,33) and, while good presumptive evidence has been provided for the solvated state, there has so far been no suitable probe for the detection of preferred rotamers in solvated glycosides. The known conformational influences upon n.m.r. spectra and, in particular, the Karplus relationship between vicinal nuclei and the nuclear Overhauser effect between stereochemically impinging resonant nuclei seemed to provide a suitable method of approach to the problem. It seems appropriate here to discuss these effects and their possible utility for this purpose.

C. Carbon-13 Magnetic Resonance Spectroscopy

The observation and measurement of carbon-13 magnetic resonances are severely hampered by the low natural abundance, low inherent sensitivity, and long relaxation times of this nucleus. Interest and activity in this field have also been limited by the high cost of isotopically enriched materials. However, recent technical advances, including the production of a number of stable field-frequency locked carbon-13 spectrometers, and the development of pulsed techniques and the required ancillary computers for Fourier transformation of the observed free-induction decay signal, have greatly improved the precision and signal sensitivity obtainable. The subject has been extensively reviewed, most recently in a monograph by Stothers (34).

1. Carbon-13 Chemical Shifts

The range of chemical shifts routinely observed in the carbon-13 spectra is exceptionally broad, being over 5000 Hz wide, compared to the 1000 Hz range of the proton spectrum. This broad range allows the unequivocal assignments of many resonances on the basis of inspection, because the shifts are so sensitive to the nuclear environment of the observed molecule. This sensitivity represents an enormous advantage of carbon-13 spectroscopy, even for natural isotopic abundance samples, and can be illustrated by comparison of the proton and carbon-13 spectra of methyl α-D-glucopyranoside. Only the anomeric and methoxyl proton signals can be assigned without careful analysis of the spin-spin coupling pattern in the proton spectrum. However, in the carbon spectrum, the resonances due to carbon-1, carbon-4, carbon-6, and the methoxyl carbon, are readily identified on the basis of chemical shift alone since they have unique chemical environments (35). Only carbons 2,3, and 5 require further analysis and carbon-3 has been identified by deuterium labelling (36).

In addition, a number of satisfactory rules can be used to aid in signal identification in a wide range of compounds (34).

2. Vicinal Carbon Proton Nuclear Spin Coupling

Early experiments involving carbon-proton coupling were carried out by observation of the proton spectra of isotopically enriched compounds. Such experiments have the natural limitation that the presence of carbon-13 to proton coupling causes increased complexity of the spectra observed. Thus, it requires careful choice of compounds with relatively simple proton spectra to avoid compounding the complex multiplicities of many-proton spectra.

TABLE 1

The Influence of Substituent Electronegativity Upon Vicinal Carbon Proton Coupling Constants (38).

Compound	$^{3}J_{^{13}C\text{-}C\text{-}C\text{-}H}$ (Hz)
Neopentyl-1-^{13}C *p*-methoxybenzoate	4.7
Neopentyl-1-^{13}C benzoate	4.8
Neopentyl-1-^{13}C *p*-nitrobenzoate	4.9
p-Methoxyphenyl pivaloate-1-^{13}C	4.6
Phenyl pivaloate-1-^{13}C	4.6
p-Nitrophenyl pivaloate-1-^{13}C	4.8
Neopentyl-1-^{13}C chloride	5.6
Neopentyl-1-^{13}C bromide	5.8
Neopentyl-1-^{13}C iodide	6.0
Pivaloyl-1-^{13}C chloride	6.0
Pivaloyl-1-^{13}C bromide	6.4

(a) Substituent Electronegativity Effects

The theoretical prediction (37), that an increase in electronegativity of substituents directly bonded to the carbon-13 nucleus should increase the value of vicinal carbon-proton coupling constants, by increasing the s-character of the ^{13}C-C bond, is borne out by the values obtained for these constants in a series of *para*-substituted neopentyl benzoates and phenyl pivalates, as shown in Table 1. However, where halogen atoms are directly bonded to the carbon-13 nucleus, observed values of vicinal coupling between carbon and proton are unusually large and vary in reverse order to that predicted on the basis of substituent electronegativity (38). When the substituted carbon is sp^2-hybridized, the effect is more substantial, as can be seen by the relative values for pivaloyl chloride and bromide.

To explain this phenomenon, Karabatsos (38) has proposed possible, through-space interactions between the proton magnetic moment and currents induced on the halogen atom by the carbon-13 atom. However, such interactions have been predicted to be small (39). In any case, both systems demonstrate that vicinal carbon-proton coupling is relatively insensitive to changes in substituent electronegativity within closely related systems, since the coupling constant changes are small in comparison to the change in substituent electronegativity. The effect of electronegative substitution at the vicinal axis atoms has been little studied in sp^3 systems, however, the influence of such substituents in sp^2 hybridized systems has been investigated by Goldstein (40,41). Karabatsos suggested that the value of the vicinal carbon proton coupling constant could be calculated by multiplying the proton-proton vicinal coupling constant, observed in an analogous system, by a factor of 0.4. When this factor is used to calculate the two-, three-, and four-bond carbon-proton coupling constants for benzene, using ethylene, butadiene, and propene, as models, near agreement is obtained (42). This suggests that there is a close behavioral relationship between carbon-proton and proton-proton systems (34).

(b) Coupling through Heteroatoms

Roberts (43,44) has shown that the value of $J_{^{13}C\text{-}X\text{-}C\text{-}H}$ depends upon the size of the heteroatom in both the five-membered aromatic heterocycles and in the tetramethyl derivatives of the group IV elements. In these compounds the size of the coupling was found to decrease with increasing size of the heteroatom.

Coupling through oxygen appears to be somewhat smaller than coupling through carbon, but shows similar, though lesser, variations with changes of substituent (34). The value of $J_{^{13}C\text{-}O\text{-}C\text{-}H}$ for dimethyl ether has been reported to be 5.4 Hz (45), which is very close to the value for the analogous $J_{H\text{-}C\text{-}O\text{-}H}$ in methanol of 5.2 Hz (46).

(c) Conformation-dependent Factors

Vicinal carbon-proton couplings decrease with increase in angles θ and θ' as defined in Fig. 8 (X=H). Karabatsos and Orzech (38) have reported that,

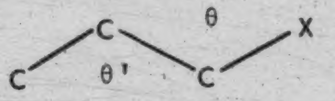


Fig. 8: The angles θ and θ' in vicinal coupling systems (38).

cases where steric factors would be expected to increase these angles, a decrease in observed coupling constant occurs. These authors support their contention that the angles θ and θ' are increased, by their observation of an unusually low carbonyl stretching frequency in the infrared spectrum of one of their compounds, di-*t*-butyl ketone. This phenomenon has been suggested to occur in all situations where large geminal angles exist (47).

Evidence of the existence of a quantitative relationship between torsion angle and vicinal carbon-13 to proton coupling has been accumulating for some time. Karabatsos (48) interpreted results of a study of temperature effects upon observed coupling, in propionaldehyde and its *O*-methyl oxime ether, as evidence that coupling was greater between nuclei in *anti*-periplanar orientation, than between those oriented *syn*-clinally. Goldstein has investigated vicinal couplings in fumaral, fumaric acid, maleic acid and maleic anhydride and found, in every case, that coupling was greater when the nuclei were in *anti*-periplanar orientation, than when they were in *syn*-periplanar orientation (40).

Perlin and Casu observed extra splittings of 5-6 Hz in the anomeric proton signal of α-glucose-^{13}C, which they attributed to coupling of this nucleus with the carbon-13 nuclei at position 3, and 5, of the pyranoid ring. These nuclei are in approximately *anti*-periplanar orientation to the anomeric proton. No similar coupling was observed in β-glucose, where these nuclei would have *syn*-clinal relative orientation (36).

Recent work of Lemieux, Nagabhushan and Paul has provided strong evidence that a quantitative relationship indeed exists between coupling and torsional angle, and that it is similar in form to the well known Karplus curve (49) of proton-proton coupling. In their work, uracil-2-^{13}C, uridine-2'-^{13}C and a number of uridine-2'-^{13}C cyclonucleosides were synthesized, and the relevant coupling constants determined. Since a variety of known torsion angles occur in these rigid cyclonucleosides, it was possible to construct a graph which exhibited the familiar $\cos^2 \phi$ curve. The coupling constant for the 90° torsional angle was determined by Lemieux and Bruce (50) who synthesized 3,4,6-tri-*O*-acetyl-1,2-(methyl-1-^{13}C-orthoacetyl)-α-D-

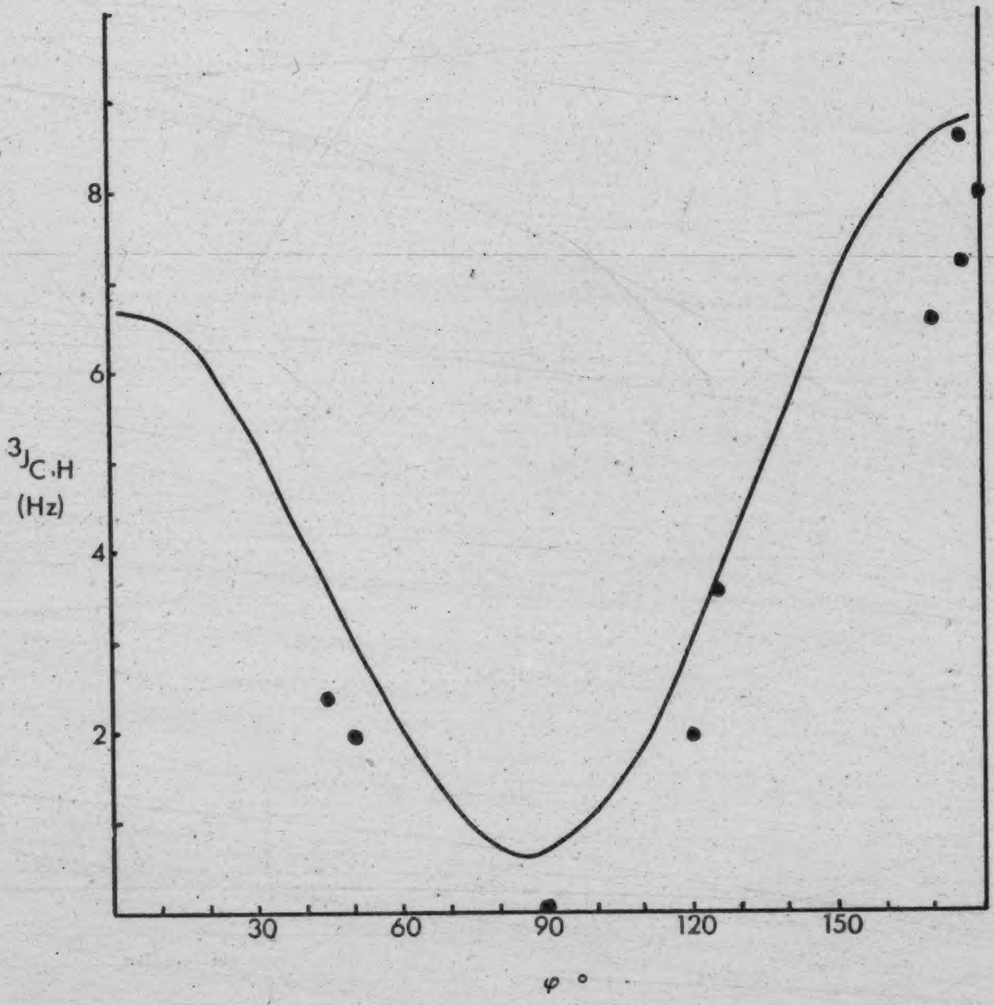


Fig. 9: The variation of vicinal carbon to hydrogen $\left({}^3J_{C,H}\right)$ with torsion angle (ϕ) obtained from INDO-MO calculations on propane (51). A plot of earlier data (●) of Lemieux, Nagabhushan and Paul (49) is included.

glucopyranose, in which the orthoacetyl carbon has a torsion angle of about 90° with both H-1, and H-2, of the pyranoid ring. The p.m.r. signals for these protons failed to show any evidence of coupling with the vicinal carbon.

Wasylishen and Schaefer have recently calculated the torsion angle dependence of vicinal carbon to proton coupling in propane, using INDO-MO formulation (51). The curve, thus calculated, is shown in Fig. 9, together with a plot of the earlier data of Lemieux, Nagabhushan and Paul. While there is clearly a satisfactory parallel between the observed coupling data of Lemieux and the calculated curve, it is also apparent, that any attempt to apply values determined from the curve to the assignment of precise bond angles, may lead to serious errors. The validity and utility of the relationship is confined to comparisons of relative bond angles in closely related systems. The equation calculated by Schaefer is

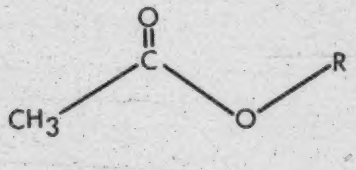
$$^3J_{C,H} = 4.26 - 1.00\cos\phi + 3.56\cos 2\phi.$$

For comparison, the Karplus equation (52), calculated for vicinal proton-proton coupling in ethane, is

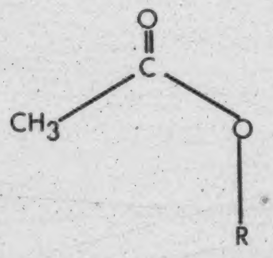
$$^3J_{H,H} = 4.22 - 0.5\cos\phi + 4.5\cos 2\phi.$$

(d) Applications in Conformational Analysis

As was noted above, there have been few reports of the application of vicinal carbon-proton nuclear spin coupling studies to conformational analysis. The first such investigation was reported by Karabatsos, Orzech and Hsi (53), in 1966. These authors carried out temperature-dependence investigations of the coupling through the ester oxygen, with the carbonyl-enriched alkyl acetates, 3 and 4.



3



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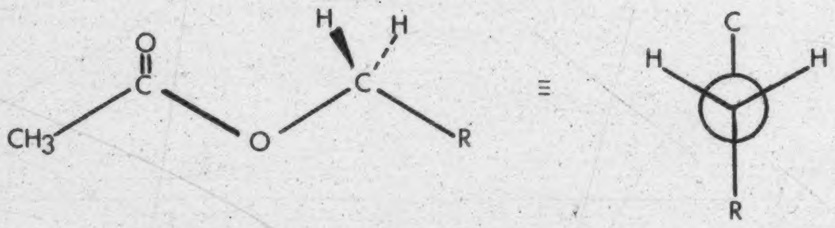
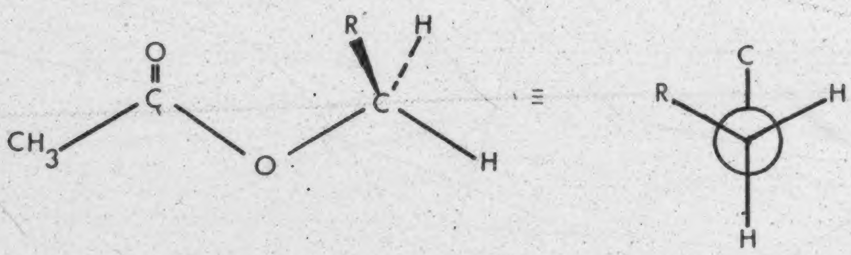
For this purpose, Karabatsos adapted the commonly-accepted mathematical relationships used in vicinal proton-proton coupling. Since similar analytical methods will be discussed in conjunction with this work, it is appropriate to review them by following the procedures reported by Karabatsos.

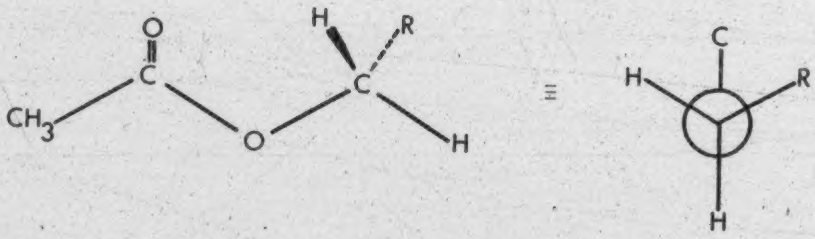
By assuming a strong preference, in acetates, for the s-*cis* rotamer, 3, over the s-*trans* rotamer, 4, the number of possible rotameric forms about the ester linkage can be reduced to the three alkyl-oxygen bond rotamers, 3a, 3b, and 3c


O
C
CH3
O
H
H
C
R
≡
C
H
H
R

3a


O
C
CH3
O
R
H
C
H
≡
C
R
H
H

3b


O
C
CH3
O
H
R
C
H
≡
C
H
R
H

3c

Inherent in this analysis is the assumption that these rotamers have the normal staggered torsion angles. Rotamers 3b and 3c are isoenergetic and, therefore, have equal populations. Since they are also equivalent with respect to coupling constant contributions, they may be treated as one rotameric species. Rotamer 3a, which has both protons in *syn*-clinal orientation to the carbon-13 nucleus, is expected to reduce the observed coupling, because of the weak coupling contribution associated with this orientation. Rotamers 3b and 3c have one proton in *anti*-periplanar, and one in *syn*-clinal orientation, with respect to the carbon-13 nucleus and, thus, would contribute a larger coupling component to the population-weighted average which is observed. The equation of relation for carbinyl acetates becomes

$$J_{obs} = nJ_g + (1-n)\,\frac{J_t + J_g}{2} \;\ldots.,$$

where n is the mole ratio of rotamer 3a and J_t and J_g are the coupling contributions expected at torsion angles of 180° and 60° respectively.

For methyl acetate, in which there are three equivalent rotamers with two *syn*-clinal protons and one *anti*-periplanar proton, the equation is further simplified to

$$J_{obs} = 1/3\,(J_t + 2J_g) \;\ldots.$$

The experimental value for methyl acetate is 4.0 Hz. However, before either equation can be evaluated, values for J_t and J_g must be determined or, as is common in vicinal proton coupling analyses, an equation relating J_t and J_g must be obtained. In the present case, Karabatsos observed that the smallest coupling constant determined in a series of acetate esters was 2.3 Hz. It was therefore considered that J_g, which was assumed to be smaller than J_t, must be smaller than this value. From the second equation, therefore, J_t must be larger than 7.4 Hz. An alternative analysis could be carried out by use of the simplified form (54) of the Karplus equation:

$$J_{obs} = A \cos^2 \phi \ldots.$$

Thus, since $\cos^2 180°/\cos^2 60° = 4$, it follows that $J_t = 4\ J_g$, and, when this information is substituted into the equation for methyl acetate, values for J_t and J_g of 8 Hz, and 2 Hz, respectively, are estimated.

Such calculations, and any analysis made from them, are clearly only approximate and, therefore, useful mainly for determining the relative energies of rotamers. In this example, when Karabatsos calculated the enthalpy differences associated with rotation around the alkyl oxygen bond of ethyl acetate, he obtained values of ΔH° ranging from 600 to 1,400 cal/mole, depending on the values chosen for J_t, and J_g. If the values of J_t and J_g calculated above from the simplified Karplus equation are used, the difference is approximately 1.3 kcal/mole.

In their paper describing the variation of vicinal coupling constant to torsional angle, Lemieux, Nagabhushan and Paul made use of the relationship to confirm the predominance of the *anti*-rotamer of uridine (49). Recently, the *anti*-rotamer of cytidine, and the *syn*-rotamer of 6-methyl cytidine, have been shown to be preferred by use of the same relationship (55).

3. Vicinal Carbon-Carbon Coupling

The exceptional improvements in carbon-13 magnetic resonance spectrometers in recent years has made possible the detection of carbon-carbon coupling in mono-enriched compounds, by observation of signals due to the normal isotope abundance carbons in the remainder of the molecule. Previously, it had been necessary to selectively enrich both molecular positions in order to achieve sufficient signal strength for observation. With the modern instruments, however, signal to noise ratios greater than 5 to 1 are readily achievable with samples in near millimolar quantity (56).

Since the improvements are only recent, there is very little published information concerning vicinal coupling. The results of several studies have been reported by Marshall and coworkers (57,58,59) including an investigation of the relationship of carbon-carbon vicinal coupling

TABLE 2

Vicinal Carbon-Carbon Coupling in Some Aromatic Derivatives

Compound	$^3J_{C-C}$ (Hz) 2-5	1-4	3-7	Reference
Toluene	—	9.5	3.8	57, 59
Iodobenzene	8.6	—	—	61
Aniline	7.9	—	—	"
Nitrobenzene	7.6	—	—	"
Pyridine	13.9	—	—	"
Benzyl alcohol	—	—	3.9	59
Benzyl chloride	—	—	4.2	"
Sodium benzoate	—	—	4.1	"
Benzoic acid	—	—	4.5	"
Methyl benzoate	—	—	4.6	"
Benzoyl chloride	—	—	5.5	"
Benzonitrile	—	—	5.8	"
Nitrotoluene	—	—	3.9	"

constants to torsion angle (60). Roberts has reported values observed in pyridine and three substituted benzenes, in which the torsional angle is 0° (62), and Marshall has investigated (59) a series of substituted benzenes, in which the torsional angle is 180°. These values are tabulated in Table 2. Values for coupling at zero torsion angle show significant variation with different substituents bonded to the coupling axis, but such variations cannot be correlated to substituent electronegativity. In the examples cited, in which coupling is between carbons at positions 2 and 5 of the phenyl ring (61), the order of size of the coupling constant and the relative electronegativity of the substituent groups, are not related. Where substituents are bonded directly to a coupled nuclei, small increases in observed coupling can be seen to be associated with the electronegativity of the substituent. As was the case in the carbon-proton coupling system (38), sp^2-hybridized nuclei are more sensitive to this influence than are sp^3-hybridized nuclei.

Marshall has reported the results of his investigations into the torsion angle dependence of this coupling (60). Using singly-enriched, rigid, alicyclic carboxylic acids, several couplings, at fixed torsion angles within the arc from 57° to 180°, were observed. A graph of the observed couplings, and the corresponding torsion angles,

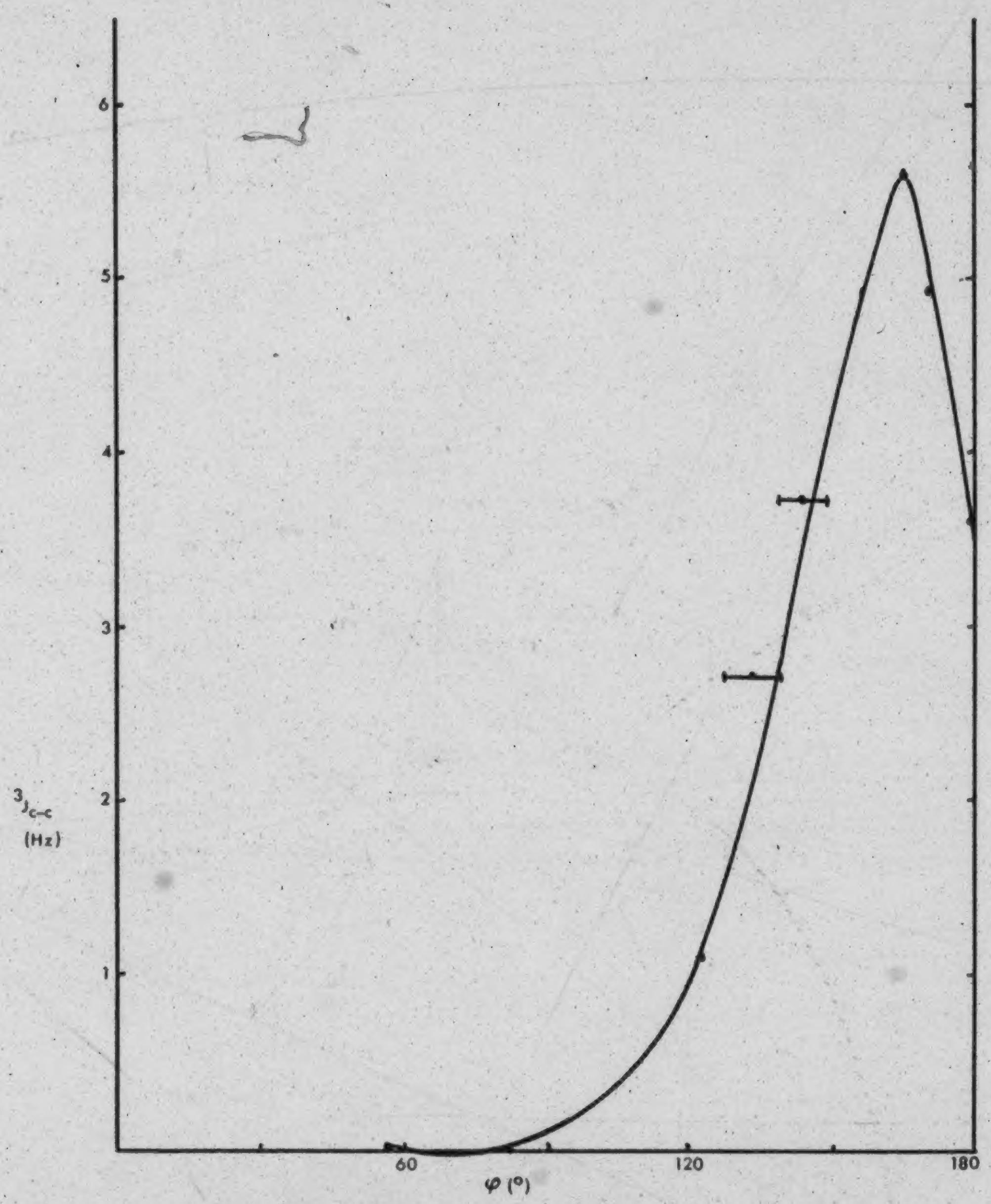
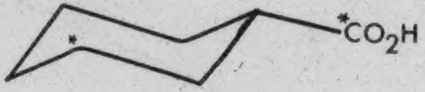
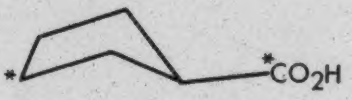
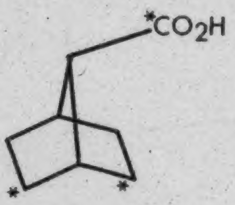
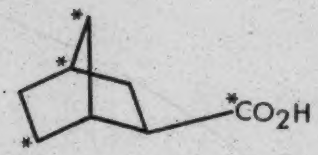
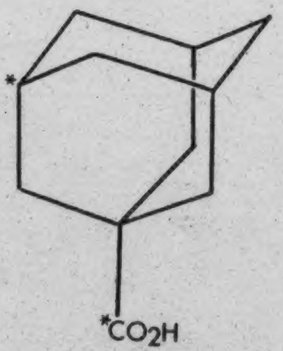


Fig. 10: The variation of vicinal carbon to carbon nuclear spin coupling with torsion angle (ϕ), (60).

TABLE 3

Vicinal Carbon-Carbon Nuclear Spin Coupling through σ Bonds (62)

Compound	φ (°)	$^3J_{C-C}$ (Hz)
$^*CH_3CH_2CH_2{}^*CO_2H$	144°	3.7
	175°	4.0
	135°	2.7
	57° 164°	0 5.6
	83° 124° 172°	0 1.7 4.9
	180°	3.6

is presented in Fig. 10. As can readily be seen, the shape of the curve, defined by the observation points, deviates somewhat from the classical $\cos^2 \phi$ form of the Karplus relationship. In particular, the observations that the region of null coupling extends at least as low as 60° of torsion, and, that the angle with greatest coupling is approximately 160° rather than 180° are exceptional. Marshall (62) has suggested a tentative explanation for these deviations, by pointing out the similarity of this curve (Fig. 10) and the theoretical fluorine-fluorine vicinal coupling curve calculated for 1,2-difluoroethane by Hirao (63). In Hirao's work, the various contributing components were also considered over the full range of torsion angles, and it could be seen that the Fermi contact contribution was the controlling factor. The compounds synthesized by Marshall and Miiler, together with their observed carbon-carbon coupling constants, are presented in Table 3.

To date, there have been no reports of similar studies involving carbons of sp^3 hybridization, so it cannot positively be stated that a similar relationship would be observed. However, it seems most likely that the relative, if not the absolute values for couplings at various angles of torsion will be similar. In this instance, observations of relative changes in size of coupling could be usefully correlated to torsion angle changes.

D. Nuclear Overhauser Effects

1. Definition and Theoretical Considerations

The nuclear Overhauser effect (n.O.e.) has been defined (64) as "a change in the integrated nuclear magnetic resonance absorption intensity of a nuclear spin when the n.m.r. absorption of another spin is saturated." The existence of this phenomenon has been recognized for some twenty years but only recently, with the discovery of its conformational utility and the availability of more sophisticated n.m.r. spectrometers, has its use become common.

While the theory (64) of the Overhauser effect is not unduly complicated, it will not be dealt with in detail here. For present purposes, it is sufficient to note that the effect of a saturating radiation at the resonance of one nuclear spin may, in certain circumstances, alter the balance of spin state populations of another species in favour of the lower energy state. As a result of this increase in lower energy spin state nuclei, the absorption capacity at this second resonance increases and, thus, the signal intensity of this species is augmented. The observation of such intensity increases is considered evidence for the close approach of the two nuclear species involved, and their magnitude has been shown to be a function of the degree of closeness of approach.

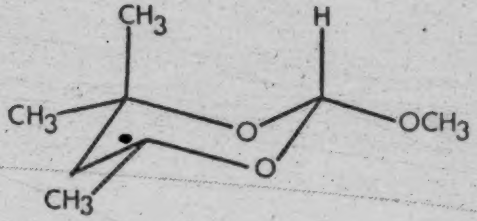
Bell and Saunders (65) have proposed the equation:

$$\text{n.O.e.} = 1/Ar^6_{AB},$$

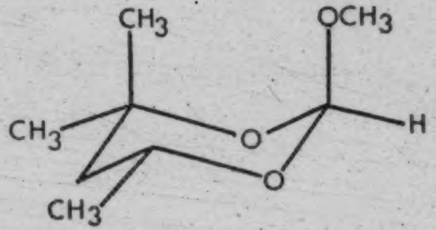
where A is a constant, to describe the dependence of the magnitude of the effect upon the internuclear separation between the irradiated species, A, and the observed species, B. This equation has been shown to be accurate until r_{AB} reaches a limiting value, at which point the n.O.e. approaches its theoretical maximum of 50%. Since the equation is based on the assumption that nuclear dipole-dipole interaction will be the dominant relaxation mechanism, it can be assumed from the good agreement that this is correct (65).

2. Applications in Structural and Conformational Analysis

An example of the qualitative utility of nuclear Overhauser effects is provided by Eliel's (67) confirmation of the structure assignments of *cis*- (5), and *trans*-2-methoxy-4,4,6-trimethyl-1,3-dioxane, (6). When the n.m.r. signal of H-2 was observed, while irradiating at the resonance of one of the singlet methyl groups attached to C-4, a 12% enhancement was noted in the compound suspected to have the *cis*-configuration, 5. No effect was observed with the compound assigned the *trans*-configuration, 6. In this manner, an assignment of configuration based upon dipole moment measurements was confirmed.


CH3
H
CH3
O
OCH3
O
CH3

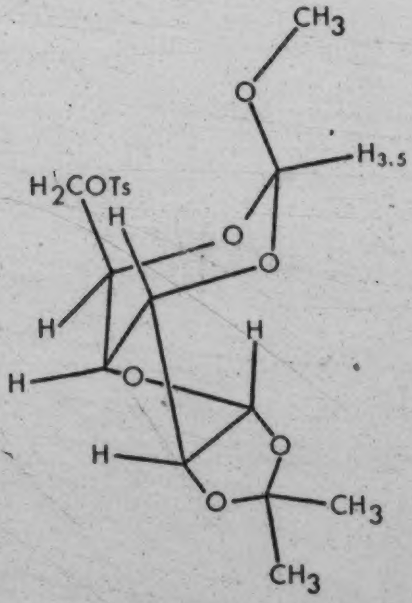
5


CH3
OCH3
CH3
O
H
O
CH3

6

Coxon (68) has used n.O.e. data to demonstrate the rotameric position of the methoxyl group in the *endo* diastereoisomer of 1,2-*O*-isopropylidene-3,5-*O*-(methoxymethylidene)-6-*O*-*p*-tolysulfonyl-α-D-glucofuranose, (7), which is shown below. A 25% enhancement of the signal for H-3,5 was noted upon irradiation at the methoxyl proton resonance.

Many similar determinations have been made which are discussed in a review article by Bachers and Schaeffer (69) and a monograph by Noggle and Schirmer (64).


CH3
O
H3,5
H2COTs
H
O
O
H
H
H
O
H
O
O
CH3
CH3

7

Among the most interesting examples of the use of nuclear Overhauser effects is their application to problems of conformational analyses about the glycosidic bond in the nucleosides, by Hart and Davis (70), and by Hart, Davis, Schirmer and Noggle (66). Enhancements of the anomeric proton signal were detected upon irradiation at the resonance of suitably located protons bonded to the purine or pyrimidine aglycon moieties. These observed enhancements were interpreted as evidence for the existence, in some proportion, of conformations previously thought to be unfavourable.

Bell and Saunders (71) have published results from their study of n.O.e. in the dimethylformamide molecule. These experiments were carried out by observation of the formyl proton and irradiation of the methyl group resonances. From observation of the relative temperature independence of the enhancement when one of the methyl groups was irradiated and the strong temperature dependence of the enhancement due to irradiation of the other methyl group, they concluded that the mere existence of an Overhauser enhancement does not imply a time-independent spatial proximity or even conformational preference but only that the two species can be located near each other for some period of time.

From the above, it can be concluded that the nuclear Overhauser effect is a useful tool in qualitative conformational analysis, and is quantitatively valid in conformationally rigid systems. For mobile systems involving internal rotation, the existence of the effect implies close approach of the species, and the magnitude of the enhancement indicates the degree of closeness obtained, but not the residence time at the spatially proximate location.

E. Optical Rotation and Molecular Conformation

1. Empirical Rules Predicting Molecular Rotation

The utility of optical rotation measurements was markedly increased by the introduction of empirical rules linking observed rotation constants with molecular configuration. Hudson developed a series of rules of isorotation which provided a fairly reliable way of distinguishing α- from β-anomers, but which were not based upon any understanding of the physical process involved (72).

The two most successful proposals for such rules are those of Whiffen (73), and Brewster (74). Modifications, simplifying the presentation of Whiffen's rules, have been proposed recently by Lemieux and Martin (75), and by Lemieux and Brewer (76). While there are differences in the types of compounds contemplated, and in the number of rules found necessary by these authors, the basic simplifying assumptions and rationalizations are quite similar. In all of these analyses, the dominant source of the generation of optical activity is considered to be conformational asymmetry. One important source of conformational asymmetry is the three bond unit, some examples of which are illustrated in Fig. 11. Such three bond units, in Brewster's rationalization, form right- or left-handed "screw patterns" of electron polarizability (74). The contribution from these interactions is correlated in sign with the handedness, such that right-handed patterns contribute positively, while left-handed patterns contribute negatively.

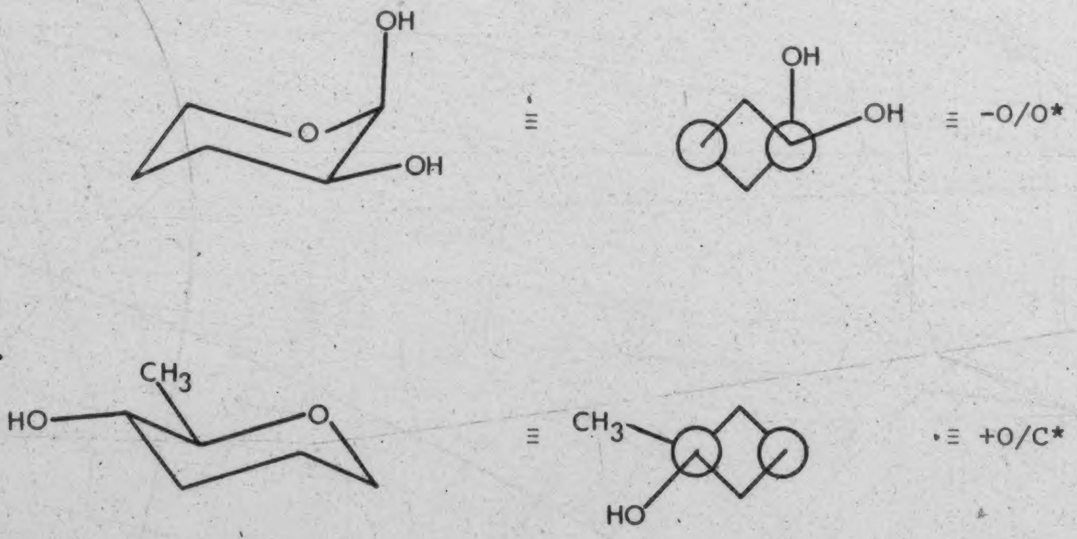


Fig. 11: Some examples of asymmetric three-bond units which contribute to optical rotation.

* This notation is the simplified version of Lemieux and Martin (75).

TABLE 4

A Comparison of the Rotation Constants Used by Whiffen, Brewster and Lemieux

Notation			Δ $[M]_D$ Assignment		
Whiffen	Brewster	Lemieux	Whiffen	Brewster	Lemieux
F	(O-H)(O-H)	O/O	45°[*]	45°	55°
G	(O-H)(O-H)	O/O	32°[*]	45°	55°
H	(C-H)(O-H)	O/C	34°	50°	45°
J	Permolecular	C_o/O	113°	100°	115°
I	Permolecular	I	43°	60°	45°
—	—	C_o/C	—	—	≥65°[†]

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[*] In contrast to the treatments of Brewster and Lemieux, Whiffen distinguished between oxygen atoms bonded to the anomeric carbon and those bonded to the other pyranoid carbons.

[†] For example, the three bond unit formed by the aglyconic carbon and C-2 of the pyranoid moiety of methyl glycopyranosides in the α-b configuration.

In all analyses the torsion angles are assumed to be the normal values of ±60° or 180°. This clearly introduces an unavoidable source of error, because the bond angles of the ring atoms of pyranoid rings are known to vary over a considerable range (77,78). The rotatory contributions of three-bond units are expected, on theoretical grounds, to be a function of the sine of the torsion angle (73) and, therefore, variations of this angle will alter the contribution of the unit. Three-bond units having the *anti*-periplanar, 180°, torsion angle are considered not to contribute to optical rotation, which is also in accord with a dependence upon the sine of this angle.

In addition to three-bond units of conformational asymmetry, all the proposed systems have included factors for an inherent asymmetry of certain substituent bond to ring oxygen configurations in the pyranoid series. These effects, termed "permolecular" by Brewster, are illustrated in Fig. 12, and the various treatments are collected and compared in Table 4.

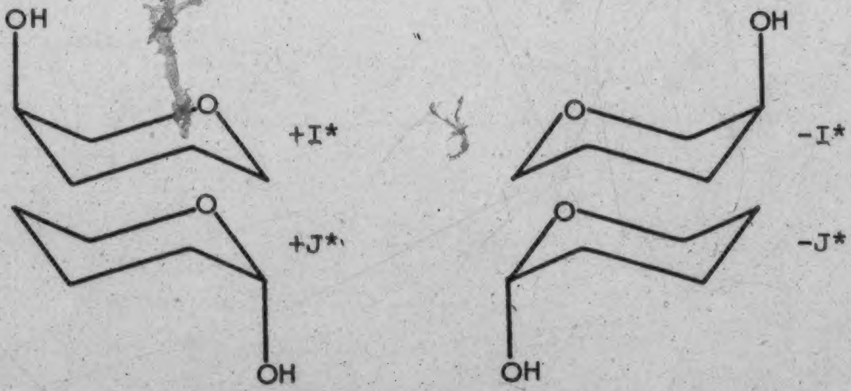


Fig. 12: Conformational units of asymmetry of pyranoid rings (permolecular effects).

*The notation is that of Whiffen (73).

Notably missing from all treatments are terms for the exocyclic hydroxyl group. Contributions from three bond units terminating with this group are assumed to be either small, or, to be made small by the averaging effect of free rotation (73,74). For the exocyclic C-6 to O-6 bond of 1,5-hexopyranoses, however, it has been found that an additional factor must be employed. Whiffen assigned a value of +30° for this source, while Brewster used +25°. Lemieux and Brewer have analyzed the contribution of this group in terms of the previously discussed three-bond units (76). All proposals have considered the problem of solvent effects to be minimal, and Lemieux has shown that conformationally rigid molecules do not show important changes in rotation when observed in different solvents (75).

2. Application of Empirical Rules to Problems of Conformational Analysis

Lemieux and Pavia have published some examples of the successful application of empirical rules for optical rotation (23). They determined the approximate mole fractions of the two ring-inversion chair conformers of a series of methyl 3-deoxy-β-L-*erythro*-pentopyranosides by comparison of the observed rotations of the two contributing conformers. The following equation was used:

$$[M_{obs}]_D = X_A[M]_{D-A} + (X_B)[M]_{D-B},$$

where X_A, and X_B, are the mole fractions, (and $X_A = 1 - X_B$) and $[M]_{D-A}$, and $[M]_{D-B}$, are the calculated molecular rotation constants of the two forms, at the D-line of sodium vapour. The results, calculated in this way, were in good agreement with those based on a similar analysis of the coupling constant between H-1 and H-2 of the pyranoid ring.

II. EXPERIMENTAL

A. Materials

1. Reagents

The starting materials tri-*O*-acetyl-1,5-anhydro-2-D-*arabino*-hex-1-enitol and penta-*O*-acetyl-β-D-glucopyranose, were obtained from Raylo Chemicals Ltd., Edmonton, Alberta. Methyl α-D-mannopyranoside and tetra-*O*-acetyl-α-D-glucopyranosyl bromide were prepared in this laboratory, using standard procedures. All starting materials were checked for purity by comparison of their physical constants with published data, and, where necessary, were recrystallized until satisfactory agreement was obtained.

Lithium aluminum hydride was purchased as a 1.04 M solution in bis-(2-ethoxyethyl) ether from Ventron Chemicals, Beverly, Mass., U.S.A., and used without further treatment. A mixture of *cis*- and *trans*-4-*t*-butylcyclohexanol was separated into the individual isomers by column chromatography, using a mixed eluting solvent consisting of benzene, isopropyl ether and ethyl acetate in 2:2:1 volume ratio. The separated isomers had melting points of 77 to 78.5° and 75.5 to 77.5°, respectively. Diborane was purchased as a one molar solution in tetrahydrofuran (Ventron Chemicals). The bromobenzene received from the supplier required extensive purification (79) to give satisfactory material of boiling range 150-155°. Methanol and barium carbonate, containing 58 moles percent carbon-13, and methyl iodide containing 62 moles percent carbon-13, were obtained from Raylo Chemicals Limited. A supply of barium carbonate, enriched to 86.8 moles percent carbon-13, was purchased from British Oxygen Co., London, England. All carbon-13 enriched materials were used as received.

2. Solvents

Reagent grade methanol was dried by distillation from magnesium methoxide according to an established procedure (80), and the distillate was stored over Linde-type 4A molecular sieves. Dichloromethane, when intended for use as solvent in glycosidation reactions, was purified by extraction with sulfuric acid according to an established procedure (79), followed by passage through a column of neutral aluminum oxide (M. Woelm A. G., Eschwege, B. R. D.) immediately prior to use. Alcohol-free chloroform, for use as the solvent in anomerization reactions, was prepared immediately prior to use by percolation through neutral aluminum oxide. Benzene, when used as the solvent for anomerization reactions, was distilled from fine sodium wire. The hexamethyl phosphoric triamide received from the supplier was dried by shaking for

48 hours over Linde type 13X molecular sieves, decanting, and then storage over a fresh batch of sieves. The 2,4,6-trimethylpyridine received from the suppliers was distilled from potassium hydroxide pellets and then stored over a few fresh pellets of the same material. Tetrahydrofuran was dried several times with sodium wire and then stored over fresh sodium wire until use. The bis-(2-ethoxyethyl) ether required for lithium aluminum hydride reductions, was dried by shaking for 48 hours with Linde type 4A molecular sieves, decanted, and then stored over a fresh batch of the same sieves.

B. Methods

1. Spectroscopy

All measurements were made by service laboratories as mentioned in Acknowledgements.

(a) Infrared Spectroscopy

All spectra were run as mulls, in Nujol, using Perkin Elmer Model 421 or 337 grating spectrometers at ambient temperature, in matched, and polished sodium chloride cells.

(b) Nuclear Magnetic Resonance Spectroscopy

i. Proton magnetic resonance spectroscopy

The proton magnetic resonance spectra were observed at 60, 100 or 220 MHz with Varian A60, A56/A60, HA-100-12, HA-100-15 and HR-220 spectrometers. Chemical shifts are reported in p.p.m. relative to tetramethylsilane (TMS) or hexamethyldisilane used as internal, or external reference standards, depending, as noted in the text, upon the solvent used for observation. When necessary, double resonance experiments, using a frequency sweep technique, were performed, to confirm the assignments of signals, and to facilitate the measurement of coupling constants. The estimated errors in reported chemical shifts and coupling constants are ±0.1 p.p.m. and ±0.1 Hz, respectively. The vicinal carbon-proton coupling constants were evaluated from 100 MHz spectra as follows.

For the alkyl glucosides, analysis of the signal for the anomeric proton was generally possible and many of the constants reported in this work were determined in this way. This analysis is simplified by several circumstances. Firstly, the difference in the chemical shifts of the coupled nuclei is much greater than the coupling constants required to be evaluated. Thus, in the symbolism generally used (81), the species involved can be treated as an AX-type system and, therefore, the coupling constants can be measured directly from

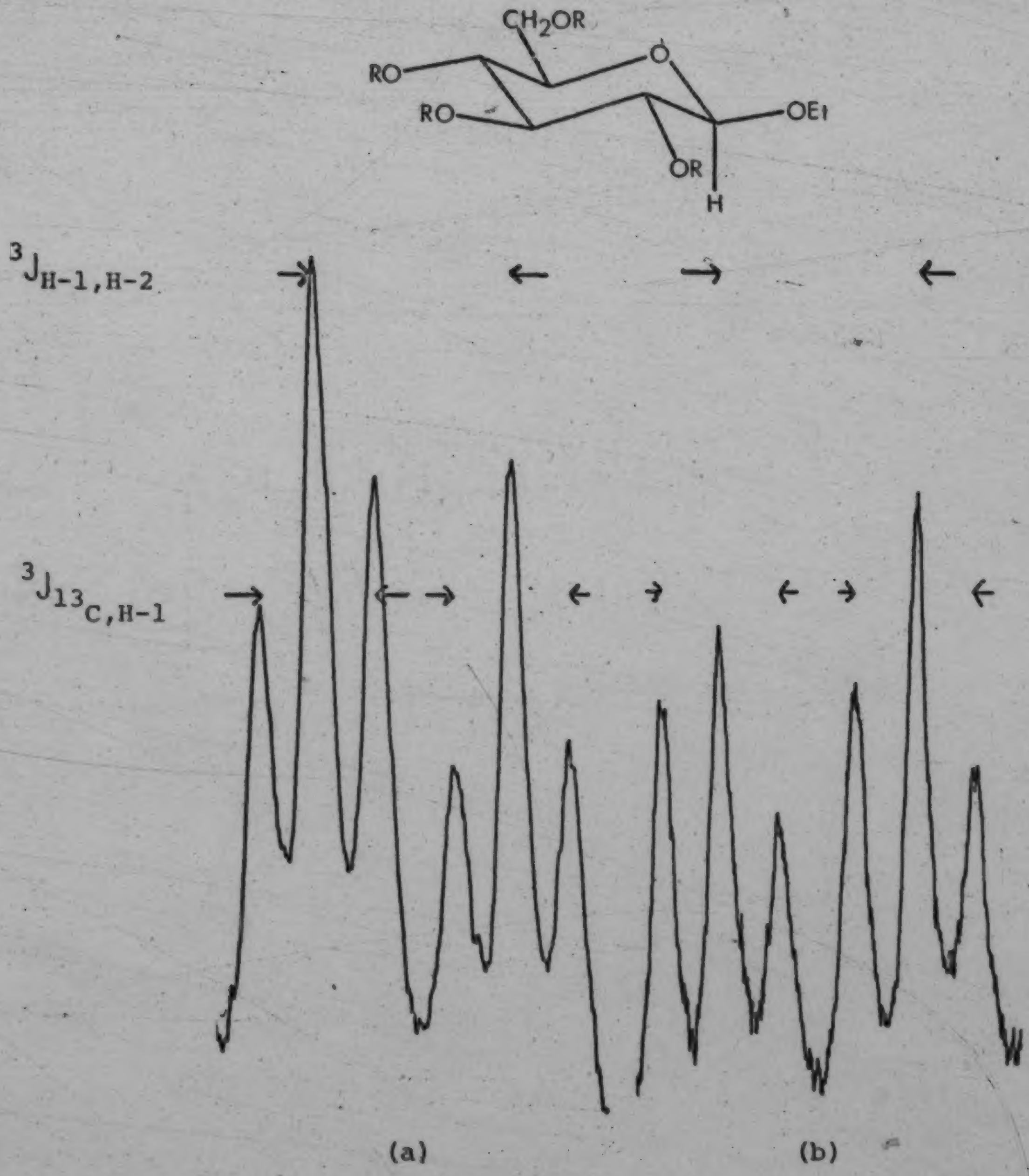


Fig. 13: The signal for the anomeric proton of ethyl-1'-^{13}C tetra-*O*-acetyl-β-D-glucoside ($\underline{9}$) in $CDCl_3$ (spectrum a) and that of the free glucoside ($\underline{8}$) in D_2O (spectrum b). The C-1' position contained 58 moles % of carbon-13.

the spectra, without recourse to mathematical analysis. Secondly, the spectra of the compounds are well-known, and the signals, therefore, were readily assigned by comparison. Finally, because the degree of carbon-13 enrichment is less than 100%, signals due to carbon-12 containing nuclei persist, and were compared with those from a normal isotopic abundance sample. The analysis of β-glucosides, in particular, is quite straightforward because of the wide spacing of the signal of the anomeric proton, due to the large coupling of H-1 with H-2. Example spectra of the signals of the anomeric protons of ethyl-1'-^{13}C β-D-glucoside (8) and its tetra-*O*-acetyl derivative (9) are presented in Fig. 13 In both cases, the total signal consists of a doublet ($J \approx$ 7.5 Hz) of doublets ($J \approx$ 4.3 Hz) centered on a doublet ($J \approx$ 7.5 Hz). Compounds 8 and 9 contained 58 moles percent carbon-13 in the aglyconic carbon, but compounds of higher levels have anomeric proton signals of slightly different appearance as can be seen in signal for *i*-propyl-2'-^{13}C β-D-glucoside, (10). which is presented in Fig. 14.

In the case of α-glucosides, $^{3}J_{H-1,H-2} \approx 3.5$ Hz, and thus, the total signal for the anomeric proton is much narrower. For ethyl-1'-^{13}C α-D-glucoside (11) the coincidence of approximately 60% carbon-13 enrichment, and the near equality of $^{3}J_{H-1,H-2}$ and $^{3}J_{C-1',H-1}$, combine to cause the signal to appear as a pseudoquintuplet as seen in Fig. 15 At higher degrees of enrichment the appearance of the signal

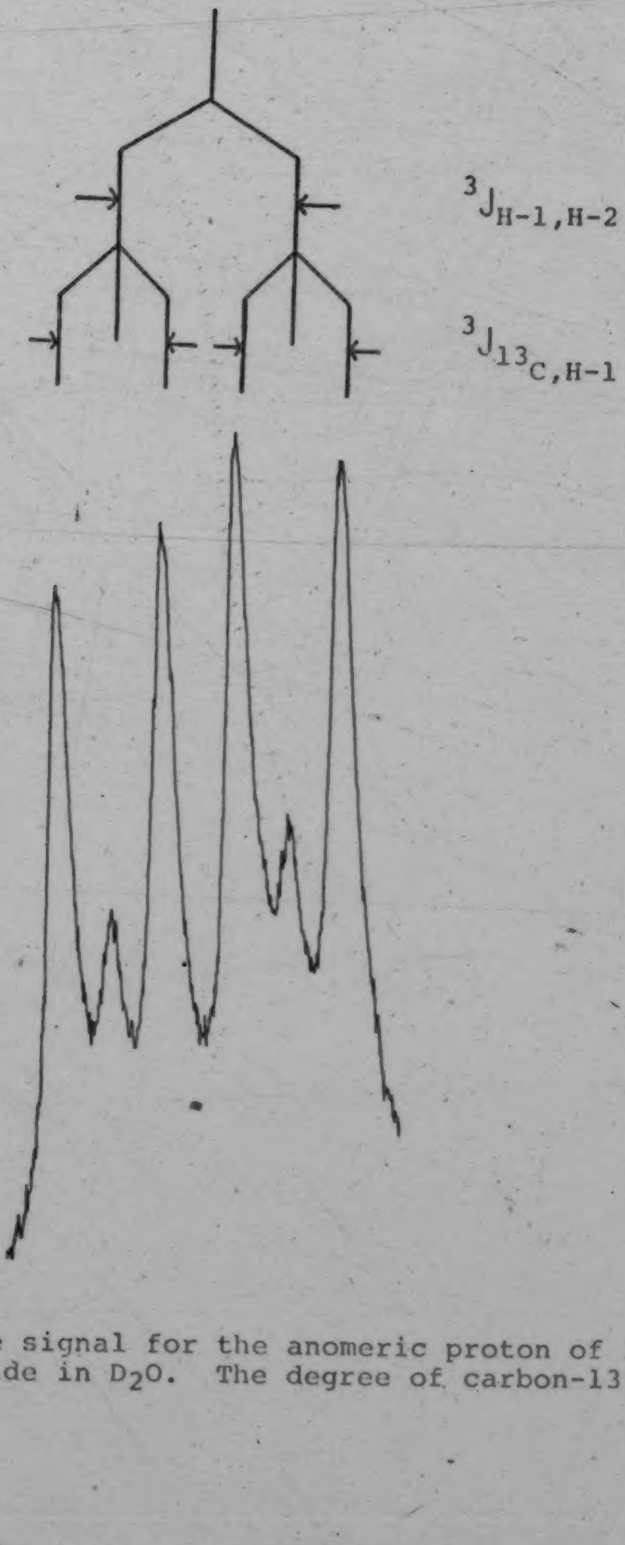
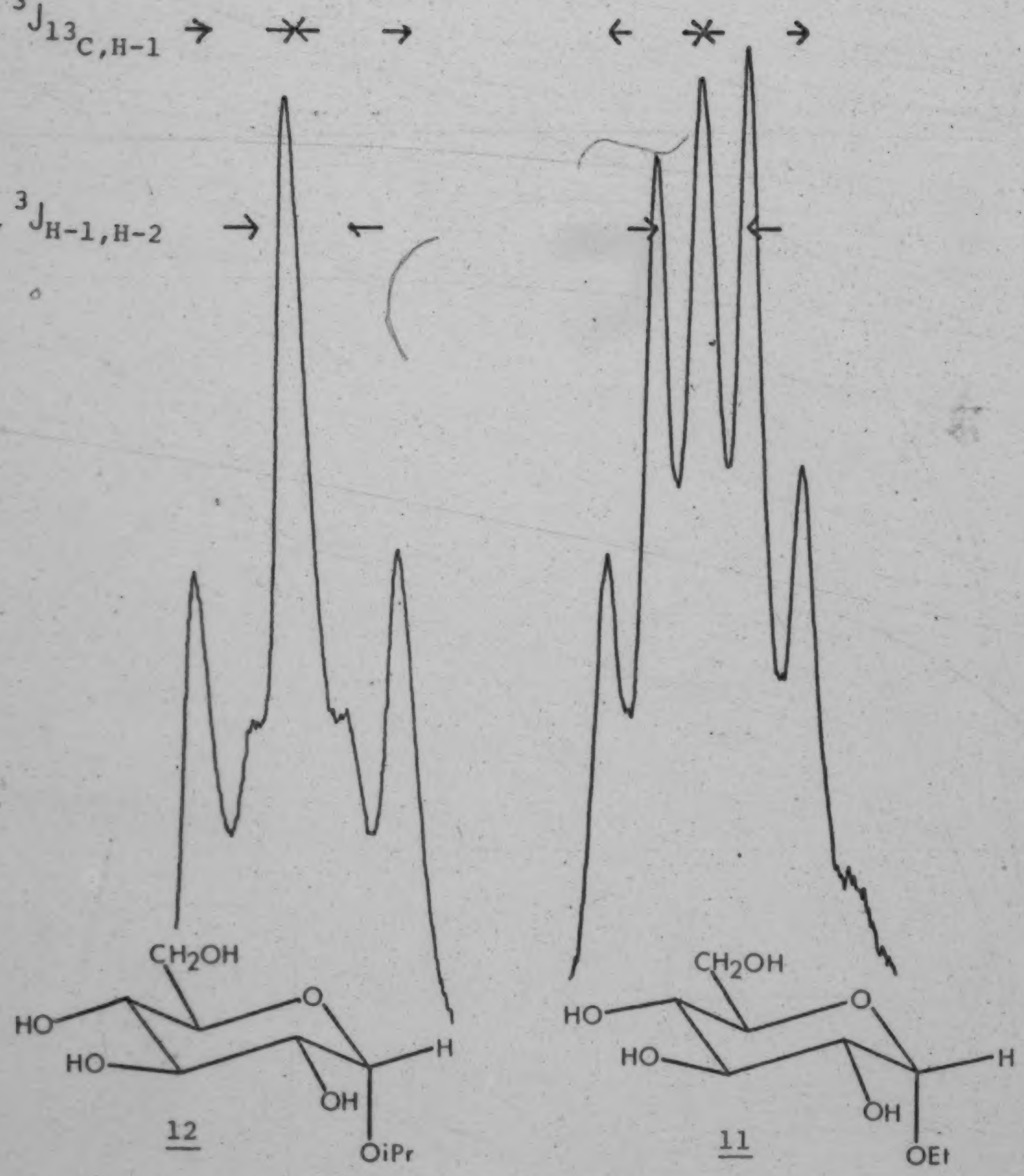


Fig. 14: The signal for the anomeric proton of isopropyl-2'-^{13}C β-D-glucoside in D_2O. The degree of carbon-13 enrichment is 88%.



12

11

Fig. 15: The signals for the anomeric protons of compounds 12 and 11 in D_2O. The degree of enrichment with carbon-13 was 88% and 58%, respectively.

of the anomeric proton changes appreciably, as demonstrated by the signal found for isopropyl α-D-glucoside (12) also shown in Fig. 15. Compound 12 contained 87 moles percent carbon-13 and, as can be seen, the central doublet due to carbon-12 glucoside is much reduced, appearing as shoulders upon the middle peak of the carbon-13 coupled signal.

Computer-assisted spectral analyses were carried out using the NUMAR program developed in this department by Dr. A. Quirt and Professor J. S. Martin (82). The computed spectrum was printed out using line printer plotting techniques and the observed plot was compared with it for similarity. Since the large chemical shift approximation (81) was valid in the case studied ($X = {}^{13}C$, $A = {}^{1}H$), only a few replications were required to simulate adequately the observed spectrum.

ii. Carbon-13 magnetic resonance spectroscopy

The carbon-13 magnetic resonance spectra were recorded at 22.63 MHz or 25.14 MHz with, respectively, Bruker HFX-90 or Varian HA-100-15 spectrometers. The Bruker instrument was interfaced to a Nicolet 1080-20K memory computer for Fourier transform analysis of the free induction decay signal observed in the pulsed mode. Pulses of 20 to 40 μs were used with a pulse length of 150 μs corresponding to a tilt angle of 90°. Initial experiments with the Varian HA-100-15 instrument were carried out by repeated scanning and then averaging the accumulated transients using a Varian-1024 computer. Later experiments were performed after the spectrometer had been interfaced to a Digilab Fourier transform system, Model FTS-NMR-3, and were carried out in the pulsed mode. Pulse widths of 30 to 42 μs were employed while the pulse width corresponding to 90° tilt angle was 50 μs.

Chemical shifts are reported in p.p.m., using the signal of the methyl carbons of TMS as standard, either externally, or internally, as noted in the text. The estimated errors for chemical shifts and coupling constants are ±0.1 p.p.m. and ±0.12 Hz, respectively, except for the carbon-carbon coupling constants, where the error is estimated to be ±0.2 Hz.

The vicinal carbon-proton coupling constants determined from carbon spectra were evaluated as follows. In the case of the methyl glycosides, the same situations responsible for the simplicity of the proton magnetic resonance spectra also serve to simplify the carbon spectra. For the higher alkyl aglycons, such as ethyl, and isopropyl, however, the increased number of protons coupled to the carbon nucleus, make the spectra too complex for precise measurement of the coupling constants involved.

Carbon spectra of compound 13 are presented in Figs. 16 and 17 All such spectra examined have a signal consisting of a quartet, (J ≈ 140 Hz), of doublets, (J ≈ 3-4 Hz).

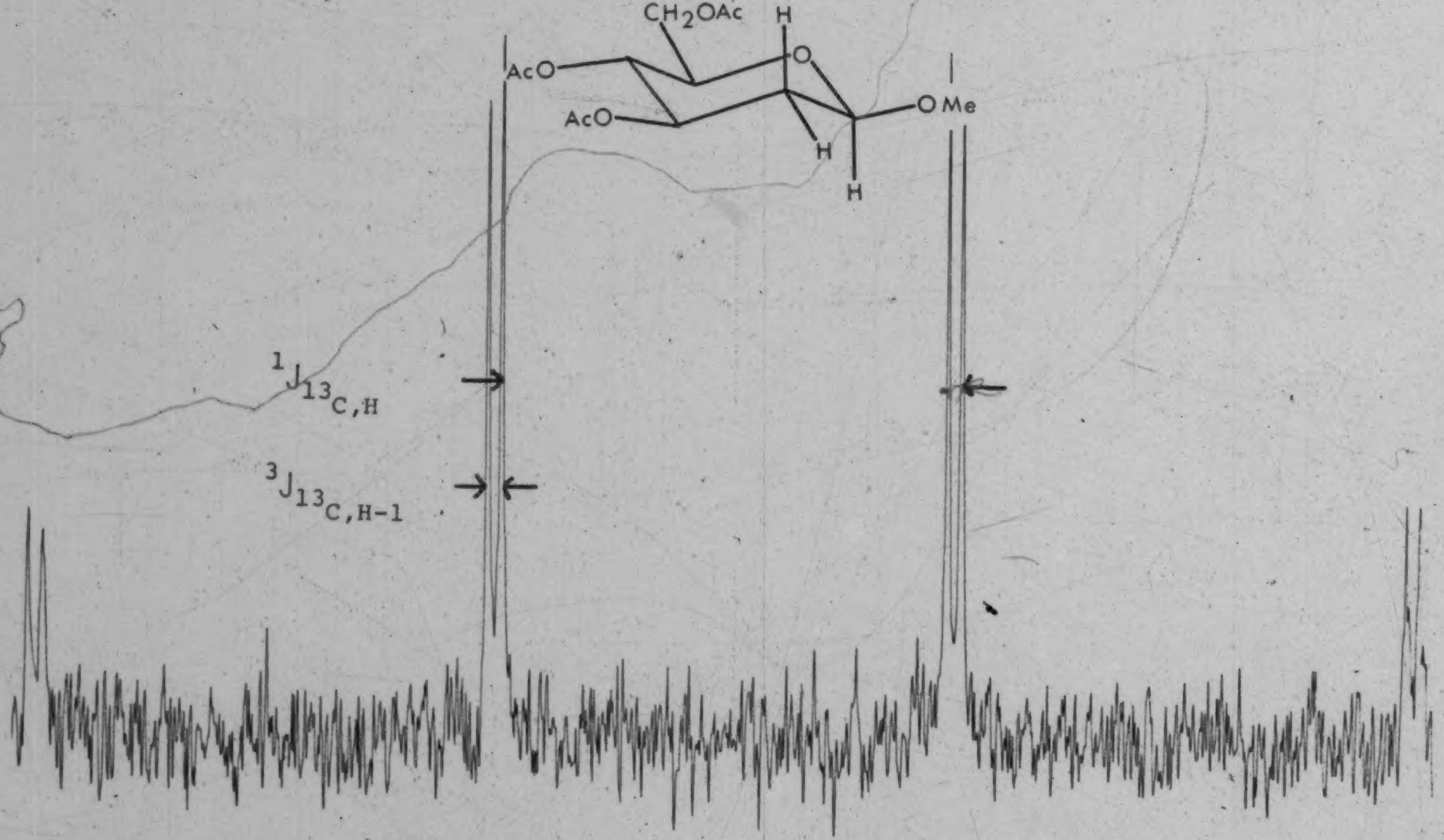


Fig. 16: The complete signal of the methyl carbon in the proton-coupled spectrum of methyl-^{13}C 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside in CDCl$_3$.

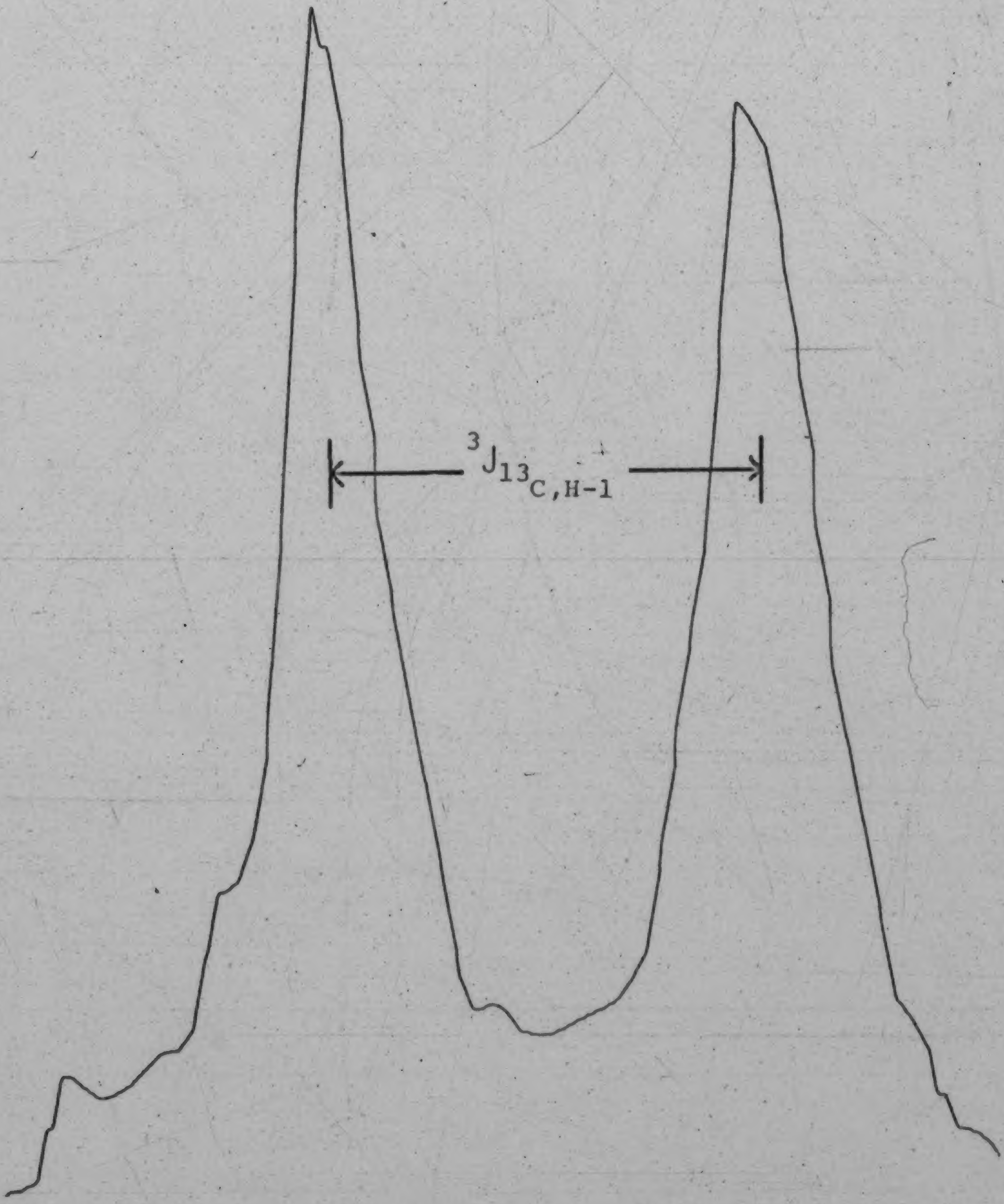


Fig. 17. An expansion to 25 Hz sweep width of an inner doublet of the proton-coupled methyl carbon signal of methyl-13C 3,4,6-tri-*O*-acetyl-β-D-*arabino*-hexopyranoside in $CDCl_3$.

Spectra observed on the Bruker HFX-90 spectrometer were evaluated by determining the computer bit number of the highest point of each peak. Since the total sweep width and the number of bits into which the sweep width was divided were known, the couplings could be determined, using the following equation:

$$J = \frac{\text{sweep width}}{\text{total computer bits}} \times \text{number of bits between signals.}$$

In practice, the sweep width observed was 500 Hz, and the number of bits used were 4096, giving a mechanical error range of ±0.12 Hz. The results of the two inner doublets were averaged and then rounded to ±0.1 Hz accuracy.

Couplings observed with the Varian HA-100-15/Digilab FTS system were measured from 25 Hz sweep width expansions of the two inner doublet signals. The estimated error in these determinations is ±0.05 Hz. The uncertainty range, arising from sweep width observed and the number of computer bits used to observe it, was 0.3 Hz, but visual measurement reduced the error to an estimated ±0.1 Hz.

(c) Mass Spectrometry

The mass spectra were measured with an Associated Electrical Industries Model MS-2, instrument.

2. Chromatography

(a) Thin Layer Chromatography

All thin layer chromatograms were carried out on Silica Gel G (E. Merck A. G., Darmstadt, B.R.D.). Various solvent systems were used for chromatogram development, depending upon the type of compound to be separated, as noted in the text. The developed chromatograms were sprayed with a variety of visualization reagents. Carbohydrates separated in this way were invariably visualized by treatment with 5% sulfuric acid in ethanol followed by heating. Non-carbohydrate materials were generally sprayed with concentrated sulfuric acid and then heated to effect charring.

(b) Column Chromatography

Except as noted, all column chromatograms were performed with 100-mesh silicic acid (Mallinkrodt Chemical Works, St. Louis, MO., U.S.A.). Columns were mounted over a mechanical fraction collector and the individual fractions were examined, either by optical rotation measurements, or by thin layer chromatography. The solvents used for elution are described in the text.

3. Optical Rotation Measurements

Optical rotation measurements were carried out using a Perkin-Elmer Model 141 automatic polarimeter operating at the D-line (589.2 n.m.) of sodium.

4. Melting Point Determinations

Melting points were determined in capillary tubes on a Gallenkamp melting point apparatus. The reported melting points are uncorrected.

5. Elemental Analyses

Elemental analyses were performed in this department by Mrs. D. Mahlow and Mrs. A. Dunn.

C. Synthetic Investigations

1. Benzoic Acid-7-^{13}C (14)

This compound was synthesized according to an established procedure (83) using 6.00 g of barium carbonate containing 58 atoms percent of carbon-13 as a source of carbon dioxide for the carbonation of phenyl magnesium bromide. Upon work up, the product was recrystallized from hot water, filtered, dried, and found to weigh 3.1 g, or 85% of theory. The white crystals had a melting point of 121.5 to 122.5°, in agreement with the reported value (79). An expansion of the p.m.r. signal of the ortho ring-portons showed an increased the multiplicity due to coupling with carbon-13 nuclei. The carbon-13 n.m.r. spectrum consisted of a triplet (J = 4.0 Hz) at 171.8 p.p.m. chemical shift. The infrared (IR) spectrum contained a sharp doublet at 1676 and 1648 cm^{-1} in the carbonyl stretching region due to a mild isotope effect which causes a slight shift in the absorption of the enriched molecules.

2. Benzyl Alcohol-7-^{13}C (15)

Benzoic acid-7-^{13}C, 3.69 g, was dissolved in a small quantity of tetrahydrofuran (THF) and the resulting solution was cooled to -30° C. Diborane, 45 ml of a 1 M solution in THF, was then added slowly by syringe. The reaction was allowed to warm to 0° C, and then kept at that temperature for 2 hours, followed by two further hours at room temperature. Methanol was added slowly until no further evolution of bubbles was noticed, and the solution was evaporated to a white viscous residue on a rotary vacuum evaporator.

The residue was dissolved in 80 ml of water and the aqueous solution was extracted with five 30 ml quantities of dichloromethane. The combined organic extracts were dried with anhydrous magnesium sulfate, filtered and evaporated to a clear residue of crude benzyl alcohol. Final purification consisted of drying by azeotropic distillation with benzene using a Dean-Stark water trap. The product, obtained after removal of the benzene, weighed 2.87 g or 88% of theory.

N.m.r. absorbances centered at 6.0, 3.7 and 2.8 p.p.m. corresponded to the aromatic, methylene and hydroxyl protons, respectively, and had the appropriate integration for benzyl alcohol. The IR spectrum was identical with that of an authentic sample, except for the existence of a sharp doublet at 1018 and 1025 cm^{-1} (C-O stretch region) in the spectrum of the carbon-13 enriched material, compared to a less distinct signal for the normal isotope sample. The mass spectrum showed major peaks at m/e = 108, 109 and 77 which were assigned to the parent ion and $[C_6H_5]+$, respectively. The carbon-13 spectrum consisted of a singlet at 163.8 p.p.m., with the protons decoupled, and a distinct triplet (J = 141 Hz) of doublets (J = 2.2 Hz) in the proton-coupled spectrum.

3. Benzaldehyde-7-^{13}C (16)

Benzyl alcohol-7-^{13}C, 2.67 g, was oxidized with CrO_3-pyridine complex, generated *in situ* according to a published procedure (84). The ether/dichloromethane solution of the product obtained from this reaction was distilled under an atmosphere of dry, de-oxygenated nitrogen and 1.92 g of benzaldehyde was obtained. The p.m.r. spectrum of this material showed increased multiplicity in the *ortho* protons of the phenyl ring and the aldehydic proton consisted of a doublet (J = 174 Hz) due to the carbon-13 containing molecules centered upon a singlet, of chemical shift 13.3 p.p.m., due to the carbon-12 molecules. The carbon-13 n.m.r. spectrum consisted of a doublet (J = 173.6 Hz) of triplets (J = 4.9 Hz), at 191.6 p.p.m., due to coupling with the aldehydic and *ortho* phenyl ring protons, respectively.

4. 2-Phenyl-5,5-dimethyl-1,3-dioxane-2-^{13}C (17)

Benzaldehyde, enriched to 58% carbon-13 in the carbonyl position, 0.24 g, was dissolved in 3 ml of dried, reagent grade benzene containing 0.23 g of 2,2-dimethyl-1,3-propanediol. The resulting mixture was added to a flask fitted to a Dean-Stark apparatus and the system was flushed with dry nitrogen. A few crystals of *p*-tolylsulfonic acid were added and the solution was refluxed for four hours under a current of nitrogen (85). The system was allowed to cool and the solution was transferred quantitatively to a separatory funnel and extracted with three 10 ml portions each of 10% aqueous sodium hydroxide and saturated aqueous sodium chloride. The organic phase was dried with anhydrous magnesium chloride, filtered and evaporated to a pale yellow syrup. The syrup was extended with a small quantity of ether, a few drops of Skelly B were added, and the solution cooled momentarily in a dry ice-acetone bath to initiate crystallization. After a few minutes, the solution was filtered rapidly, dried and the weight was determined to be 0.37 g. The crystalline product melted at 33 to 34°, upon recrystallization. The p.m.r. spectrum of the carbon-13 enriched material showed several changes from that of a normal sample. The acetal proton signal was a doublet ($J = 159.2$ Hz) centered on a singlet of chemical shift 5.2 p.p.m., due respectively to the carbon-13 and carbon-12 molecules in the isotopic mixture. The signals of the axial and equatorial protons were affected to different degrees; that of the equatorial protons showing a large coupling ($J \approx 8$ Hz) with the carbon-13 nucleus at C-2, while the signal of the axial proton was broadened slightly due to minor couplings ($J \approx 1$ Hz) with the carbon-13 nucleus. Computer-assisted analysis of the 220 MHz spectrum gave values of 7.8 and 0.9 Hz, respectively, for the carbon-13 coupling constants of the equatorial and axial protons.

5. *cis*-Methoxy-^{13}C-4-*t*-butylcyclohexane (18)

cis-4-*t*-Butylcyclohexanol, 0.25 g, was dissolved in 2.5 ml of hexamethyl phosphoric triamide and a small quantity of sodium hydride was added. The reaction mixture was cooled in an ice bath and 0.11 ml of methyl iodide (containing 62 atoms percent carbon-13) was added by syringe.

The reaction mixture was left stirring at room temperature for 36 hours, at which time it was shown to be complete by t.l.c. (hexane:ether, 4:1). The reaction vessel was cooled in ice, and methanol was added slowly, with stirring, until no further evolution of gas could be detected. The resultant solution was partitioned between hexane and water, and the hexane was dried with anhydrous $MgSO_4$. The hexane was distilled off, and the residue was mixed with a small quantity of chloroform, charged upon a silicic acid column, and eluted with chloroform. Fractions were collected, and analysed by t.l.c. by comparison with an authentic sample. Those containing the desired product were collected, and the chloroform was distilled off. The carbon-13 n.m.r. spectrum of this compound showed only one absorption, of chemical shift 55.7 p.p.m., and consisted of a quartet (J = 140 Hz) of doublets (J = 3.7 Hz), as expected.

6. *trans*-Methoxy-^{13}C-4-*t*-butylcyclohexane (19)

trans-4-*t*-Butylcyclohexanol, 0.25 g, was treated as for the *cis*-isomer, and worked up as before. The ^{13}C-n.m.r. spectrum for this compound consisted of a quartet, (J = 140 Hz), of doublets (J = 3.78 Hz), at 55.8 p.p.m.

7. Silver Di-sym-collidine Perchlorate

Silver nitrate, 9.0 g, and sodium perchlorate, 11 g, were reacted with 20.0 ml of sym-collidine according to a published procedure (86). The yield obtained was 2.4 g, approximately quantitative.

8. Iodonium Di-sym-collidine Perchlorate

This compound was prepared and analysed according to a published procedure (86). The yield was 101.4% of the theoretical based on the formula $(C_8H_{11}N)_2IClO_4$.

9. Methyl-^{13}C 3,4,6-Tri-*O*-acetyl-2-deoxy-2-iodo-α-D-mannopyranoside and the Corresponding -β-D-Glucoside

Anhydrous methanol (containing 58 atoms % carbon-13), 0.25 ml, was reacted with 1.56 g of D-glucal triacetate and 2.69 g of iodonium di-sym-collidine perchlorate according to the procedure of Lemieux and Morgan (86) but with the further precaution of excluding all light. The p.m.r. spectrum of the syrupy product thus obtained showed anomeric methoxyl proton signals consisting of two doublets ($J \approx 145$ Hz) due to the ^{13}C-bound protons, centered on two singlets of chemical shift 3.42 and 3.54 p.p.m. due to ^{12}C bound protons. By integration, the ^{13}C-enrichment was confirmed to be 58%. The yield obtained was 2.33 g, 93%.

10. Anomeric Methyl-^{13}C 3,4,6-Tri-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranoside

The syrupy mixture of methyl^{13}C 3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-D-manno- and -glucopyranosides, obtained above, was dissolved in a small quantity of ethyl acetate containing 1.1 ml of diethylamine and 0.5 g of palladium catalyst on charcoal support. The mixture was hydrogenated at atmospheric pressure until no further hydrogen was absorbed and then filtered, and partitioned between toluene and water. After several extractions, the toluene solution was dried with anhydrous magnesium sulfate and evaporated on a rotary evaporator. The thick syrup, thus obtained, was further evaporated to constant weight, under high vacuum conditions. The yield obtained was 1.61 g or 93%, calculated from D-glucal triacetate. The p.m.r. spectrum (C_6D_6) of this material contained the expected methoxyl proton signal, consisting of two doublets, ($J = 145$ Hz), due to protons bonded to ^{13}C atoms, centered on two singlets, of chemical shift 3.88, and 4.05 p.p.m. due to ^{12}C-bonded protons.

11. Separation of the Anomers of Methyl-^{13}C 3,4,6-Tri-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranoside

The syrupy anomeric mixture, obtained above, was mixed with a small quantity of isopropyl ether, and charged upon a narrow, dry-packed column of Silica Gel G, 96 g, which was then eluted with a mixed solvent made up of six volumes of isopropyl ether and one volume of ethyl acetate. The eluate was collected, using a rotary fraction collector set to collect fractions of 10 ml, and each such fraction was analysed by observation of its optical rotation. The most dextro-rotatory fractions were combined and evaporated to constant weight. The p.m.r. spectrum in $CDCl_3$, of the pale, yellow syrup, had the methyl proton signals in the form of a pseudotriplet made up of a doublet, (J = 144 Hz), centered on a singlet at chemical shift 3.88 p.p.m., due, respectively, to the carbon-13 and carbon-12 containing molecules. The signal of the anomeric proton was highly complex, and the coupling with the aglyconic carbon was determined in a double resonance experiment to be approximately 3.5 Hz. This fraction was identified as the α-anomer by comparison of the n.m.r. spectrum with that of an authentic sample. The weight of product was 0.69 g, obtained as a syrup which had $[\alpha]_D$ 119.9° (c 1,$CHCl_3$). Fractions of weaker optical rotation were compared by thin layer chromatography using the same solvent system as that used for the column chromatography and those fractions containing essentially no faster running spots were combined and evaporated under vacuum. The syrupy material crystallized readily from ethanol. The yield was 0.23 g of a product which melted at 96-97° [lit. m.p. 97-99°] (87). By comparison of the p.m.r. spectrum with that of an authentic sample, the product was identified as the β-anomer. The methoxyl proton signal was a doublet, (J = 144 Hz), centered on a singlet, of chemical shift 4.05 p.p.m. in benzene-d_6. The absorbances of the anomeric proton were complex and showed a new spacing of approximately 4.3 Hz.

12. Methyl-^{13}C 2-Deoxy-α-D-*arabino*-hexopyranoside (21)

Methyl-^{13}C tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside, 0.69 g was dissolved in 10 ml of dry methanol and 1.1 ml of triethylamine was added. The resulting solution was kept at 50° C for 14 hours at which time complete deacetylation had occurred as shown by t.l.c. (4:1, chloroform:methanol). The solution was evaporated, under vacuum, in a rotary evaporation apparatus and the thick, near-crystalline mass was recrystallized from acetone giving 0.38 g of a product which melted at 90-92°, [lit. m.p. 92-93°] (87). The n.m.r. spectrum of this compound showed the expected pseudotriplet methoxyl proton signals, (J = 145 Hz), with chemical shift of 5.87 p.p.m., and increased spacings in the signal of the anomeric proton (J = 3 Hz) due to coupling with the aglyconic carbon.

13. Methyl-^{13}C 2-Deoxy-β-D-*arabino*-hexopyranoside (22)

Methyl-^{13}C tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside, 0.23 g, was dissolved in 10 ml of dry methanol containing 1.1 ml of triethylamine. The resulting solution was kept at 40° C for 14 hours at which time deacetylation was shown to be complete by t.l.c. The mixture was worked up as before and on crystallization, a product of m.p. 120-122° [lit. m.p. = 122-123°] (88) was obtained. The p.m.r. of this product, in D_2O, exhibited the expected pseudotriplet signal for the methoxyl protons (J = 144 Hz) with a chemical shift of 6.17 p.p.m. The signal of the anomeric proton occurred as a quartet reflecting coupling constants of 4.6 and 8.6 Hz, and was centered on a doublet (J = 8.6 Hz), due to the ^{13}C-containing and ^{12}C-containing molecules, respectively.

14. Methyl-^{13}C Tetra-*O*-acetyl-β-D-glucopyranoside (23)

Penta-*O*-acetyl-β-D-glucose, 1.20 g, was dissolved in 24 ml of dry benzene containing 0.12 ml of methanol (58%-^{13}C) and the solution was heated to 40°. Stannic chloride, 0.18 ml, was dissolved in 6 ml of dry benzene and the resulting solution was added to the reacton mixture which was then left for one hour (89). The reaction mixture was extracted with cold water, saturated aqueous sodium bicarbonate, and twice more with cold water. The benzene solution was then dried with anhydrous magnesium sulfate, filtered, and evaporated on a rotary evaporator. A small quantity of methanol was added to the thick syrup, the mixture was seeded and then refrigerated. The crystalline material, of melting point 104° [lit. m.p. 105°], was obtained in total yield of 0.58 g, and had $[\alpha]_D$ -18.2 (*c* 1,$CHCl_3$), [lit. $[\alpha]_D$ -18.7] (90).

The p.m.r. spectrum for this compound contained methoxyl proton resonances consisting of a doublet, ($J_{^{13}C-H}$ = 143 Hz), centered on a singlet of chemical shift 3.49 p.p.m. and, also, showed increased multiplicity in the signal of the anomeric proton (J = 4.3 Hz) due to coupling with the aglyconic carbon.

15. Methyl-^{13}C 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside (24)

Methyl-^{13}C 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, 0.32 g, was dissolved in 4.45 ml of purified chloroform and 4.45 ml of a 0.2 M chloroform solution of titanium tetrachloride was added. The reaction vessel was sealed and heated at 61° C for 2 hours (91). The mixture was then extracted with saturated aqueous sodium bicarbonate and twice with water. The chloroform was dried with anhydrous magnesium sulfate, evaporated, and left overnight under high vacuum. The syrupy product was dissolved in 1 ml of ethanol, and the solution was seeded with an authentic sample and refrigerated for 4 hours.

The crystalline product, 0.21 g, had $[\alpha]_D$ 130.1° (*c* 1,$CHCl_3$) [lit. $[\alpha]_D$ 134.4°] and melted at 100° C [lit. m.p. = 101-102°] (90). The p.m.r. spectrum, which had satisfactory integration, was compared to that of an authentic sample and found to be identical except for the methoxyl- and anomeric-proton signals which showed increased multiplicity due to the presence of the ^{13}C-containing molecules. The p.m.r. spectrum of this compound contained methoxy-proton resonances consisting of a doublet (J = 147 Hz) centered upon a singlet with a chemical shift of 2.99 p.p.m. (C_6D_6) and also showed increased multiplicity in the anomeric proton signal (J = 3.5 Hz).

16. Methyl-^{13}C β-D-Glucopyranoside (1)

Methyl ^{13}C 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside, 0.140 g, was dissolved in dry methanol, 10 ml, and a sliver of metallic sodium was added. After five minutes, the reaction was found to be complete by t.l.c. The reaction mixture was stirred with a small quantity of IR-120 H^+ cationic resin, until neutral to pH paper, and was then filtered. After several washings of the resin with methanol, the combined filtrate was evaporated on a rotary evaporator. The thick syrup was further evaporated, under high vacuum, to constant weight. The yield was 0.7 g or 91%. After recrystallization, the product had a melting point of 103°, [lit. value 108°] and $[\alpha]_D$ -32.8

(*c* 1, H_2O), [lit. $[\alpha]_D$ -34.2] (93). The n.m.r. spectrum was identical with that of an unenriched, authentic sample except for the methoxyl signal which consisted of a doublet, (J = 144 Hz), due to ^{13}C bound protons, centered on a singlet, with a chemical shift of 4.05 p.p.m., due to ^{12}C bound protons and, also, the anomeric signal, which consisted of a quartet (J = 4.6 Hz), centered on a doublet (J = 7.4 Hz) due, respectively, to the ^{13}C- and ^{12}C-containing molecules.

17. Methyl-^{13}C α-D-Glucopyranoside (25)

Methyl ^{13}C tetra-*O*-acetyl-α-D-glucopyranoside, 0.130 g, was dissolved in 10 ml of dry methanol and a sliver of metallic sodium was added. The reaction mixture was treated as for the β-anomer and crystals, 0.061 g, m.p. 166° [lit. m.p. 166°], $[\alpha]_D$ 157.2, (*c* 1, H_2O) [lit. $[\alpha]_D$ = 158.9°] (94), were obtained. After deuterium exchange of hydroxyl protons by means of repeated lyophilization, the p.m.r. spectrum was found to have the expected signals and multiplicities. The signals for the methoxyl proton consisted of a doublet (J = 145 Hz) centered upon a singlet, whereas the signal of the anomeric proton occurred as a pseudo-quintuplet made up a triplet (J = 3.8 Hz), due to coupling with the aglyconic carbon and H-2, centered upon a doublet (J = 3.6 Hz) due to coupling with H-2 only. The carbon-13 n.m.r. spectrum had one signal, a quartet (J = 144.6 Hz) of doublets (J = 3.8 Hz) due, respectively, to coupling with the methoxyl and anomeric protons.

18. Methyl Tetra-*O*-benzyl-α-D-mannopyranoside (26)

Methyl α-D-mannopyranoside, 10 g, was dissolved in 100 ml of HMPT, and 98 ml of benzyl chloride were added to the mechanically stirred solution. Sodium hydride, 10 g, washed free of its paraffin oil coating with Skellysolve B, was then added, in small quantities, over a 5 minute period. After some slight frothing and rapid temperature rise with the initial additions, further quantities resulted in only slight temperature increases, levelling off at 45° C. The reaction mixture was heated to 65° and kept there for 4 hours. The progress of the reaction was then checked by t.l.c., (Skellysolve B:ethyl acetate, 4:1), and found complete. The cooled reaction solution was filtered to remove excess sodium hydride, extended with 200 ml of water, and the aqueous mixture was extracted with Skellysolve B until no carbohydrate material could be detected (by charring with sulfuric acid) in the HMPT-water phase. The combined extracts were evaporated to a thin, lachrymatory syrup, which was subjected to steam distillation to remove the benzyl chloride which remained. The resulting syrup was decanted from the hot water and evaporated to a thick syrup which resisted all attempts to induce crystallization. The p.m.r. spectrum of this material gave a satisfactory integration and showed absorbances corresponding to aromatic, benzylic, pyranoid and methoxyl protons. The yield was quantitative.

19. 2,3,4,6-Tetra-*O*-benzyl-D-mannopryanose (27)

Compound 26, 28 g, was dissolved in 600 ml of glacial acetic acid, and 435 ml of 11% aqueous sulfuric acid was added to the stirred solution. The resulting mixture was heated on a steam bath, with continuous stirring, for 17 hours, at which time no starting material could be detected by t.l.c. (benzene:hexane:ethyl acetate, 2:2:1). The cooled solution was extended with 200 ml of water and extracted with dichloromethane. The organic phase was, in turn, extracted with saturated, aqueous sodium bicarbonate, then water, and finally dried with anhydrous sodium sulfate. The crude mixture, obtained on evaporation of the dichloromethane solvent, was chromatographed through a column of silicic acid, using the same solvent eluent as was used above for t.l.c. The yield of syrupy product, obtained by evaporation of the solvents from the product fractions, was 8.0 g from 21.0 g of the mixture. The p.m.r. spectrum of this product had a satisfactory integration and exhibited resonances consistent with the expected structure.

20. 2,3,4,6-Tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-α-D-mannopyranose (28)

Tetra-*O*-benzyl-D-mannopyranose, 8.0 g, was dissolved in 40 ml of pyridine containing 3.3 g of *p*-nitrobenzoyl chloride. The system was flushed with nitrogen and then refluxed for two hours under a nitrogen atmosphere. After cooling, the reaction solution was poured onto 300 ml of crushed ice and stirred for 30 minutes. A thick semi-crystalline paste which separated from the cold aqueous phase was dissolved in 100 ml of dichloromethane and combined with two further extracts from the ice water mixture. The combined organic solutions were extracted with aqueous sodium bicarbonate and water, and then dried with anhydrous sodium sulfate. The solvent was evaporated and crystalline material was obtained from ether-hexane. The recrystallized material existed as soft, pale yellow crystals of melting point 105° and $[\alpha]_D$ 51.3° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{41}H_{39}O_9N$: C, 71.41; H, 5.57; N, 2.03.

Found: C, 71.39; H, 5.57; N, 2.26.

This represents the first synthesis of this compound. The n.m.r. spectrum exhibited the resonances expected for the structure assigned and had a satisfactory integration.

21. 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl Bromide (29)

Compound (28), 1.0 g was reacted with hydrogen bromide in dichloromethane, essentially according to a procedure reported in the literature (94). The reaction was worked up in the usual way and evaporated. The syrupy product was not characterized further, due to reported instability of the glucose analogue, but instead, was reacted immediately, as reported below. The yield was not determined.

22. Methyl-^{13}C Tetra-*O*-benzyl-β-D-mannopyranoside (30)

Methanol, 0.4 ml of a sample containing 58 moles percent carbon-13, was added to an ether slurry, containing 1 g of Drierite and 1 g of silver carbonate, and stirred for 30 minutes. Tetra-*O*-benzyl-α-D-mannopyranosyl bromide (29), dissolved in a small quantity of ether, was added slowly, over 15 minutes, and the mixture was left stirring overnight. After the usual work up, the pale syrup was crystallized from ether-Skellysolve B, to give a product which had a melting point of 67.5 to 68.5° and $[\alpha]_D$ 61.6° (*c*, $CHCl_3$). The yield was 0.43 g, 48%. The p.m.r. spectrum of this compound had satisfactory integration and showed the expected signals for this structure. The methoxyl proton had a pseudotriplet signal (J = 142.6 Hz) arising from the carbon-12-carbon-13 isotopic mixture present in the sample.

Anal. Calcd. for $C_{35}H_{38}O_6$: C, 75.78; H, 6.90.

Found: C, 75.90; H, 6.94.

23. Methyl-^{13}C Tetra-*O*-acetyl-β-D-mannopyranoside (31)

Compound 30, 0.34 g, was reacted under Birch reduction conditions, following a procedure described in the literature (95). The yield of crude crystalline material, obtained from ether-Skellysolve B, was 0.20 g, or 90%. Upon recrystallization a product which melted at 160° [lit. m.p. 161°] (90), was obtained. The p.m.r. spectrum had satisfactory integration and the resonances expected for the structure assigned. A comparison of the p.m.r. spectrum with that of an authentic sample showed them to be identical except for the increased spacings in the methoxyl and anomeric proton signals. The carbon-13 n.m.r. spectrum, with protons decoupled, consisted of a singlet of chemical shift 57.3 p.p.m. in $CDCl_3$, while the coupled spectrum was a quartet (J = 143.5 Hz) of doublets (J = 4.4 Hz).

24. Methyl-^{13}C β-D-Mannopyranoside (32)

Compound 31, 0.2 g, was deacetylated, according to a standard procedure, using aqueous methanol and triethylamine. A crystalline product, which had a melting point of 73-75° [lit. m.p. 74-75°] (96) was obtained. The p.m.r. spectrum of this product was compared with that of an authentic, non-enriched sample and found to be identical except for the increased spacings found in the signals of the methoxyl and anomeric protons. The carbon-13 n.m.r. spectrum, in D_2O, consisted of a quartet (J = 144.1 Hz) of doublets (J = 4.3 Hz), at chemical shift 56.8 p.p.m. (calculated from $\delta_{TMS} = \delta_{acetone} + 30.2$ p.p.m.) (34).

25. Methyl-^{13}C Tetra-*O*-acetyl-α-D-mannopyranoside (33)

Compound 31 was treated with titanium tetrachloride following a published procedure (89). The recrystallized product, which melted at 63-64° [lit. m.p. 65°], had $[\alpha]_D$ +48.8 (*c* 1, $CHCl_3$), [lit. $[\alpha]_D$ +49.1] (97). The p.m.r. spectrum of this product had satisfactory integration and was found to coincide with that of an authentic sample except for the extra spacings expected from the presence of carbon-13. The carbon-13 spectrum, in $CDCl_3$, consisted of a quartet (J = 143.1 Hz) of doublets (J = 3.9 Hz) at chemical shift 55.3 p.p.m.

26. Methyl^{13}C α-D-Mannopyranoside (34)

Compound 33 was deacetylated with aqueous triethyl amine-methanol according to a standard procedure. The crystalline product had a melting point of 192° [lit. m.p. 193°] and $[\alpha]_D$ +77.2 (*c* 1, H_2O) [lit. value +79.2] (96). The p.m.r. spectrum of this compound was identical with that of an authentic sample except for the extra couplings with carbon-13. The carbon-13 spectrum exhibited a quartet (J = 144.1 Hz) of doublets (J = 3.8 Hz).

27. Sodium Acetate-1-^{13}C (35)

A solution of 120 mmoles of methyl magnesium iodide was reacted according to an established procedure (98) with the carbon dioxide released from 6.0 g of barium carbonate containing 58 mole percent of carbon-13. Upon work up and titration to the methyl red indicator end-point with sodium hydroxide the solution was filtered and freeze dried. The yield was 2.33 g, 94.8%, based on barium carbonate. A sample of this product gave a p.m.r. spectrum consistent with pure sodium acetate but showed a further splitting (J = 6.0 Hz) due to the presence of carbon-13.

28. Acetic Acid-1-^{13}C (36)

Acetic acid-1-^{13}C was prepared by reacting sodium acetate-1-^{13}C with gaseous hydrogen chloride, in a gas-solid reaction system, by following an established procedure (99). The yield, from 1.1 g of sodium acetate, was 0.73 g, 93% of theory. The p.m.r. spectrum of this compound showed it to be of high purity and to have the correct integration for acetic acid. Since the spectrum was taken of a neat sample of material, prepared from non-enriched barium carbonate, no extra coupling was observed.

29. Acetyl-1-^{13}C Chloride (37)

Acetic acid-1-^{13}C, 2.9 g, was reacted with phthaloyl chloride according to an established procedure (98). The yield of clear colourless product was 3.3 g or 89% of theory. The n.m.r. spectrum, of a sample prepared from non-enriched acetic acid, was identical with that of an authentic sample and showed the product to be of high purity.

30. Ethanol-1-^{13}C (38)

Acetyl chloride-1-^{13}C, 1.05 g, was reduced with lithium aluminum hydride according to an established procedure (98). The yield of clear colourless product was 0.6 g, or 98% of theory. The n.m.r. spectrum, of a non-enriched sample of this compound prepared in the same manner, showed the expected signals for pure ethanol.

31. *t*-Butanol-2-^{13}C (39)

Acetyl-1-^{13}C chloride, 3.3 g, was reacted with an ether solution of methyl magnesium iodide, 85 m.moles. The acetyl chloride was distilled *in vacuo* into a flask containing the Grignard reagent. After some time, distillation had effectively ceased, due to the formation of a permanent gas arising from the reaction of residual hydrogen chloride with the Grignard reagent. At this point, both flasks were frozen in liquid nitrogen and the system was evacuated. The reaction flask was isolated from the system and warmed to -10° C. After 40 minutes, dry nitrogen was admitted, the vessel was fitted with a reflux condenser, and the reaction mixture was refluxed for one hour. The cooled mixture was poured into 10 ml of 4N sulfuric acid and 50 ml of crushed ice. The two layers were introduced into an apparatus suitable for continuous extraction of aqueous solutions by ether and the aqueous layer was extracted for 28 hours. The ether was largely separated from the product by distillation, at ambient pressure, using a spinning band apparatus with controllable reflux ratio. The yield, as determined by gas chromatography, was 1.8 g or 56%, based on acetyl chloride. The n.m.r. spectrum, of a sample synthesized using non-enriched materials, showed the product to consist of 60% *t*-butanol and 40% diethyl ether.

32. Barium Acetate-1-^{13}C (40)

Methyl magnesium iodide was reacted with carbon dioxide essentially as described (98) for the preparation of sodium acetate-1-^{13}C, except that the product acetic acid was titrated with crystalline barium hydroxide octahydrate. The yield, on the basis of 6.0 g of enriched barium carbonate, was 3.45 g or 46%. The n.m.r. spectrum of this compound showed the expected doublet due to carbon-13-containing acetate centered on a singlet due to the carbon-12 acetate.

33. Acetone-2-^{13}C (41)

Barium acetate, 6.0 g, enriched with carbon-13, was placed in a quartz flask and heated, *in vacuo*, to 150° for two hours to remove any traces of water present. A trap, fitted with a vacuum stopcock, was connected to the vacuum system and evacuated. When the pressure fell below 0.01 mm of mercury, the pump was isolated from the system and the trap was cooled with liquid nitrogen. At this point, a Bunsen burner, adjusted to give a pale blue flame, was directed at the quartz flask containing the barium acetate. The contents frothed slightly for about 15 minutes during which time it was heated and shaken slightly. After the reaction was complete the trap was found to contain a quantity of silicon grease due to volatilization of the super-heated acetone. The acetone was separated from this contaminant by means of a simple distillation, *in vacuo*. The p.m.r. spectrum of a neat sample of non-enriched acetone, obtained in a similar manner, showed the expected singlet at approximately 2 p.p.m., and no other signals in appreciable amounts. The yield was 1.0 g, 74%.

34. Isopropanol-2-^{13}C (42)

Acetone-2-^{13}C, 1.0 g, was reduced with lithium aluminum hydride by following, essentially, the procedure described (99) for the reduction of acetyl chloride to ethanol. The yield was 1.0 g, 97%. The p.m.r. spectrum had the expected resonances and integration.

35. Ethyl-1'-^{13}C Tetra-*O*-acetyl-ß-D-glucoside (9)

A slurry consisting of ground, anhydrous calcium sulfate, 6.7 g, mercuric oxide, 2.2 g, mercuric bromide, 0.2 g, and 30 ml of dry, alcohol-free dichloromethane was degassed in the usual way by alternate freezing in liquid nitrogen, vacuum pumping, and thawing. Ethanol-1-^{13}C, 0.6 g, was distilled into the frozen mixture. The flask was removed from the vacuum line, warmed to room temperature and dry nitrogen gas was admitted. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide, 6.0 g, was dissolved in a small quantity of the purified dichloromethane and injected into the stirred reaction mixture in one portion. After 12 hours, the reaction was found to be complete by t.l.c. (Skellysolve B: ethyl acetate, 2:1) and the reaction product was worked up, first by filtration to remove the solids and extraction of the filtrate, several times, with water. Then the dichloromethane solution was dried over anhydrous sodium sulfate and evaporated to a thick syrup. The syrup was extended with 95% ethanol prior to seeding with an authentic sample of the product. The crystalline material was filtered, dried and weighed. The yield was

2.9 g, 61% based on the ethanol, and the recrystallized product melted at 106° [lit. m.p. 106.8°] (100). The p.m.r. spectrum was identical with that of an authentic sample except that the signal of the anomeric proton was coupled to the aglyconic carbon (J = 4.4 Hz) and the aglyconic proton signals had a large coupling of approximately 140 Hz.

36. Ethyl-1'-^{13}C β-D-Glucopyranoside (8)

This material was prepared by deacetylation of the tetra-*O*-acetyl derivative, 9, with aqueous methanol-triethylamine. Crystalline material which melted at 88 to 90° [lit. m.p. 90.4°] (100) was obtained from ethyl acetate. The p.m.r. spectrum was identical with that of an authentic sample with the exception of the increased multiplicity in the signals of the ethyl group and the anomeric proton. The anomeric proton had extra spacings (J = 4.6 Hz) due to coupling with the aglyconic carbon.

37. Ethyl-1'-^{13}C Tetra-*O*-acetyl-α-D-glucoside (43)

This material was prepared from the β-anomer (9) by treatment with titanium tetrachloride following a standard procedure (101). The product was recrystallized from 70% aqueous ethanol to a material which melted at 61-62° [lit. m.p. 58-59°] (102) and with $[\alpha]_D$ +133.2° (*c* 1, $CHCl_3$) [lit.

$[\alpha]_D$ +132°] (100). The p.m.r. spectrum was identical with that of an authentic sample but with the extra spacings expected because of the isotopic mixture present. The anomeric proton had a coupling constant of 3.9 Hz with the aglyconic carbon.

38. Ethyl-1'-^{13}C α-D-Glucopyranoside (11)

Compound 43 was deacetylated with sodium methoxide in dry methanol following a standard procedure. The reaction was worked up by neutralization with IR-120H$^+$ resin and the neutral solution was filtered and evaporated to a syrupy material which was extended with ethyl acetate prior to refrigeration to induce crystallization. Crystalline material, which melted at 113-114° [lit. m.p. 114.6°] (100), was obtained. The p.m.r. spectrum was identical with that of an authentic sample except for the expected increase in multiplicity due to coupling with carbon-13. The anomeric proton exhibited a coupling (J = 3.8 Hz) with the aglyconic carbon.

39. *t*-Butyl-2'-^{13}C Tetra-*O*-acetyl-β-D-glucoside (44)

A mixture of ground calcium sulfate, 3.3 g, mercuric oxide, 1.1 g, and mercuric bromide, 0.1 g, was stirred for 30 minutes with an ether solution containing 1.8 g of *t*-butanol-2-^{13}C. A total of 6.5 g of tetra-*O*-acetyl-α-D-glucopyranosyl bromide was added over several hours. After the usual work up, 1.3 g of crystalline material which melted at 143-145° [lit. m.p. 145°] (103) was obtained. The mother liquors were concentrated and subjected to column chromatography (benzene:ethyl acetate, 4:1; 60 g of silicic acid). A further yield of 0.3 g was obtained giving a total yield of 12% based on the *t*-butanol. The p.m.r. spectrum of this material was identical with that of an authentic sample except for the increased coupling from the presence of carbon-13 nuclei. The anomeric proton signal exhibited coupling to the aglyconic carbon (J = 4.0 Hz).

40. *t*-Butyl-2'-^{13}C β-D-Glucopyranoside (45)

This compound was prepared by deacetylation of 44, 0.6 g, using sodium methoxide in methanol. The yield of crystalline material, which melted at 163-165° [lit. m.p. 164-166°] (103), was 0.33 g, 94%. When compared to that of an authentic sample, the p.m.r. spectrum of this compound was found to be identical except for the increased coupling due to the presence of carbon-13 nuclei. The signal for the anomeric proton exhibited a spacing (J = 4.2 Hz) from coupling with the aglyconic carbon.

41. Isopropyl-2'-^{13}C Tetra-*O*-acetyl-β-D-glucoside (46)

Isopropanol-2-^{13}C, 1.0 g, was reacted with 5.5 g of tetra-*O*-acetyl-α-D-glucopyranosyl bromide, using the mercuric oxide-mercuric bromide method previously described. The recrystallized product, 3.9 g, 61%, melted at 136-137° in accord with the reported value (104). The p.m.r. spectrum of this compound had the expected absorbances and a satisfactory integration. The coupling constant for vicinal aglyconic carbon to anomeric hydrogen coupling was found to be 4.7 Hz.

42. Isopropyl-2'-^{13}C β-D-Glucopyranoside (10)

Compound 46, 1.2 g, was deacetylated with sodium methoxide in methanol. Upon recrystallization from ethyl acetate, material which melted at 128° [lit. m.p. 128°] (103), was obtained in 89% yield, 0.61 g. Comparison of the p.m.r. spectrum with that of an authentic sample showed them to be identical except for extra spacings in the isopropyl and anomeric proton signals. The value of the coupling constant, between the aglyconic carbon and anomeric proton, was determined to be 4.6 Hz.

43. Isopropyl-2'-^{13}C Tetra-*O*-acetyl-α-D-glucoside (47)

This compound was synthesized by treatment of the β-anomer, 46, 1.4 g, with titanium tetrachloride to effect anomerization, as previously described. The product, 1.0 g, 72%, was recrystallized from 95% ethanol and had m.p. 84-86° [lit. m.p. 86-88°] (105). The p.m.r. spectrum of this compound was identical, except for couplings with carbon-13, with that of an authentic sample. The vicinal aglyconic carbon to anomeric proton coupling constant was found to be 4.2 Hz.

44. Isopropyl-2'-^{13}C α-D-Glucopyranoside (12)

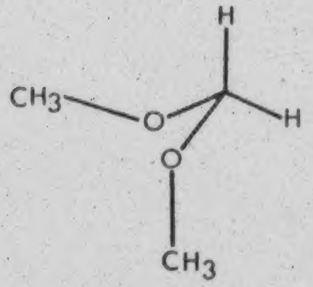
Compound 47, 0.6 g, was deacetylated with sodium methoxide in methanol to give a pale yellow syrup, 0.32 g, 94%, which resisted attempts at crystallization. The p.m.r. spectrum, allowing for the carbon-13 content, compared well with that of an authentic sample and exhibited no impurities. The vicinal aglyconic carbon to anomeric proton coupling constant was 3.9 Hz.

III. DISCUSSION

A. Vicinal ^{13}C-H Coupling-Model Compounds

As was mentioned in the Introduction, considerable information has now accumulated on the relationship of the coupling constant, $^{3}J_{C,H}$, for carbon-13 and hydrogen atoms separated by three sigma bonds (that is, in vicinal relationship) and the torsion angle that these atoms define. In the early stages of this work, however, much of this information was not available and, since an important aspect of this work was to attempt to utilize an expected Karplus-type relationship for the study of rotamer populations about the glycosidic bond, it was therefore considered necessary to reinforce the then available information on this subject by studies of appropriate models.

Four model structures were chosen, namely, compounds 2, 17, 18 and 19.



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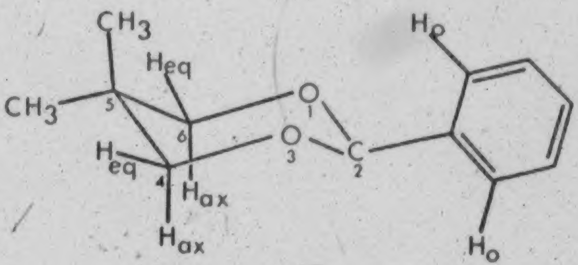
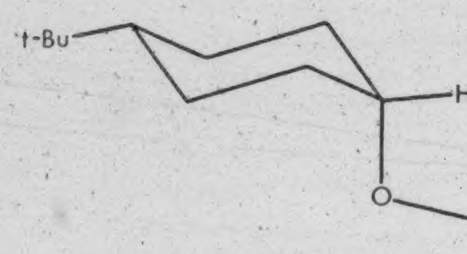
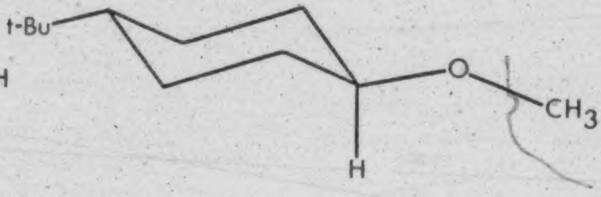


17



18



19

For compound 17, measurement of the coupling between ^{13}C-2 and the two equivalent axial hydrogen nuclei, H^4_{ax} and H^6_{ax}, would provide an estimate of the coupling between vicinal nuclei which define a torsion angle in the region of 60° through a carbon-oxygen axis. Similarly, measurement of the coupling constant between ^{13}C-2 and the two equivalent equatorial hydrogen nuclei, H^4_{eq} and H^6_{eq}, would provide similar information in a system defining a torsion angle of approximately 180°. As will be seen below, these coupling constants were found to be,

$$J^{\sim 60°}_{C,H} = 0.9 \text{ Hz}$$

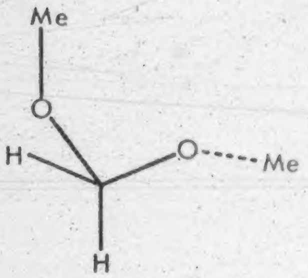
$$\text{and} \quad J^{\sim 180°}_{C-H} = 7.8 \text{ Hz.}$$

These values provided evidence that a Karplus-type relationship does exist for coupling between vicinal carbon and hydrogen nuclei in this system.

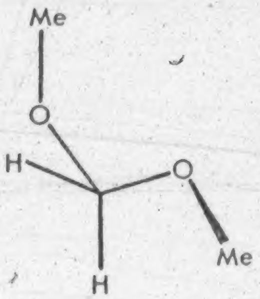
Compound 17 was synthesized by the following route. Barium carbonate, enriched to 58 moles percent with carbon-13, was used to generate carbon dioxide for the carbonation of phenyl magnesium bromide. The resulting benzoic acid was reduced with diborane to benzyl alcohol and the alcohol was subsequently oxidized to benzaldehyde. The enriched benzaldehyde was then condensed with 2,2-dimethyl-1,3-propane-diol to yield 17.

Compounds 18 and 19, which have structures in part analogous to the α- and β-glycopyranosides, respectively, were prepared by methylation of the corresponding *cis*- and *trans*-4-*t*-butylcyclohexanols. Methyl iodide, enriched to 62 atoms per cent carbon-13, was used so that the coupling could be determined by carbon-13 n.m.r. The experimental values for the coupling constants in 18 and 19 were found to be 3.7 and 3.8 Hz, respectively. For the *cis*-isomer, at least, it is certain that the methyl group must be virtually entirely in the *syn*-clinal orientation with H-1 because of the extreme steric interactions which would result if the *anti*-periplanar orientation were adopted. In the case of the *trans*-isomer, although the *anti*-periplanar orientation is somewhat less sterically unfavourable, it is still expected that the methyl group will, to a large degree, adopt the *syn*-clinal orientation. The close correspondence of the two coupling constants is in agreement with this expectation. If it is assumed that the average torsion angle defined by the methyl group and H-1 is 60°, then the value for $J^{60°}_{C,H}$ appears to be much larger in 18 and 19 than that found for the dioxane 17. The problem of correspondence between geometrically similar but constitutionally different structures such as these, is undoubtedly complex. As mentioned previously in the Introduction, a variety of factors are known to influence coupling constants (34,38). Of these factors, substituent group electronegativity and torsion angle appear most important. In this case, the effect of small torsion angle changes is especially great because the regions of inflection in the Karplus curve occur at 60° and 120°. While there exists no precise and quantitative treatment for situations of this kind, it nevertheless seems valuable to attempt a consistent, quantitative interpretation for purposes of discussion, provided that it is clearly understood that the interpretation is approximate and arbitrarily based. In this thesis, values derived from quantitative treatment of data are considered to indicate trends rather than to represent absolute physical constants.

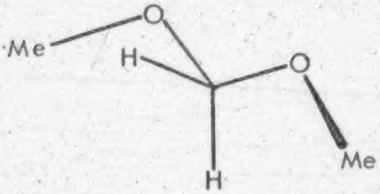
Of the model compounds examined, the vicinal carbon-13 to hydrogen coupling constants found for methylal, (2), are considered most appropriate for use in the development of a quantitative treatment suitable for application to alkyl glycosides. Firstly, methylal contains the essential acetal feature of methyl glycopyranosides and, secondly, there exists much evidence that this compound adopts the doubly *syn*-clinal orientation (2a) to an overwhelming degree. This conclusion arises, as previously mentioned, from experimental determinations of its structure by electric dipole moment (15)



2a



2b



2c

and electron diffraction (16) studies and from arguments based in studies of the *endo*- (24) and *exo*-anomeric (14) effects. This conclusion is of importance with respect to observations, to be discussed later, regarding the orientation of the methyl group in methyl glycopyranosides. For this reason, the first approximation used in the development here of a quantitative treatment of observed coupling constant data is to assume that conformers 2b and 2c have negligible populations. The second approximation made for this purpose is that the *syn*-clinal torsion angles are 60°. Astrup, on the basis of his electron diffraction studies, reported that the torsion angle defined by the methyl carbon and vicinal oxygen atom of 2 was 63° (16). The assumption that 2 assumes ideal 60° torsion angles is not likely to be exactly correct (2) because the space requirement of the C-O group on one side is probably not the same as that of the C-H bond on the other side of the preferred arc. The angle reported by Astrup is in accord with this observation since the resultant methyl carbon to hydrogen torsion angle would be expected to be 57°. The third approximation, required as a basis for pursuing discussion, is to assume that the coupling constant for a torsion angle of 60° is one-quarter that at 180°. This assumption follows from the least sophisticated form of the Karplus equation (52), as expressed by Booth (54); that is,

$$J = K \cos^2 \phi,$$

where K is the coupling constant for torsion angles at either 0° or 180°. On the basis of these assumed approximations, the following interpretation is made of the observed average coupling of the carbon-13 in the methyl groups of methylal, (2), with the methylenic hydrogens. In conformer 2a both methyl groups are equivalent and each has one vicinal proton in *syn*-clinal orientation and one in *anti*-periplanar orientation so that

$$^3J_{C,H} = \frac{J^{60°}_{C,H} + J^{180°}_{C,H}}{2} .$$

The experimental value of this coupling in $\underline{2}$ is 6.6 Hz, and, therefore,

$$J^{60°}_{C,H} + J^{180°}_{C,H} = 13.2,$$

and since, $J^{60°}_{C,H} = 1/4\ J^{180°}_{C,H}$, it follows that

$$J^{60°}_{C,H} = 2.6 \text{ Hz and } J^{180°}_{C,H} = 10.6 \text{ Hz.}$$

Support for this conclusion is provided by the vicinal carbon-13 to hydrogen coupling found in dimethyl ether. Assuming only staggered conformations for this compound, the average coupling constant should be

$$J_{obs} = \frac{2\ J^{60°}_{C,H} + J^{180°}_{C,H}}{3},$$

$$= \frac{2 \times 2.6 + 10.6}{3} = 5.3 \text{ Hz}$$

In fact, the value found by Dreeskamp (45) is 5.4 Hz. Therefore, these values will now be used to derive a Karplus expression of the form (106),

$$^3J_{C,H} = A + B\cos\phi + C\cos 2\phi.$$

This expression requires that A - B + C = 10.6 Hz and, since $J^{90°}_{C,H} \approx 0$, A ≈ C. Karplus (106) has used a value of -0.5 for the constant B in ethane, which implies that

$$^3J^{180°}_{C,H} = {}^3J^{0°}_{C,H} + 1.$$

If that value is applied to the carbon-hydrogen coupling system, and the explicit equations,

$$^{3}J^{60°}_{C,H} = 2.6 = A - 0.5B - 0.5C, \text{ and}$$

$$^{3}J^{180°}_{C,H} = 10.6 = A + 0.5 + C,$$

are solved, then values for A and C of 5.3 and 4.8, respectively, are obtained. This expression yields a value of only 0.5 Hz for $^{3}J^{90°}_{C,H}$. The assumption that B is -0.5, as proposed for a vicinal proton coupling system by Karplus, is arbitrary, but seems reasonable considering the general similarity of the carbon-hydrogen and hydrogen-hydrogen coupling systems, previously commented upon (38).

As mentioned above, this expression is not expected to yield precise and correct quantities, but rather, it is considered useful to indicate likely trends in the change in coupling constant with change in torsion angle as observed in the course of this work and especially as applied to carbon-13 to anomeric hydrogen coupling in methyl-enriched glycopyranosides. Fig. 18 presents a plot of this Karplus-type expression in order to demonstrate the rapid change in coupling constant which occurs in the region of 60° of torsion. The values of $^{3}J_{C,H}$ for torsion angles of 50°, 60° and 70° are 4.2, 2.6 and 1.4 Hz, respectively. This wide variation of coupling constant, within the narrow range of torsion angles of 50 to 70°, is most likely to be the reason

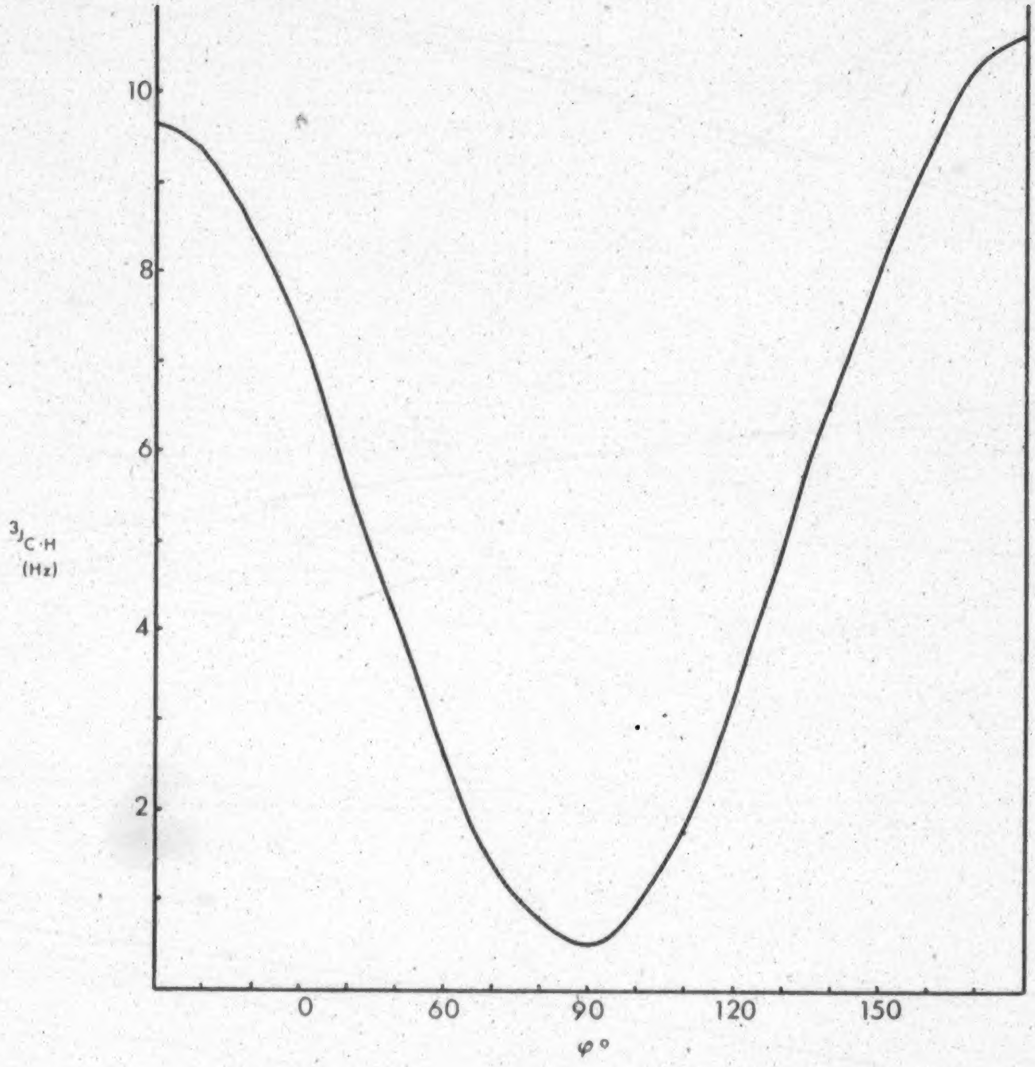


Fig. 18: Plot of $^3J_{C,H}$ versus torsion angle, ϕ, for the relationship $^3J_{C,H} = 5.3 - 0.5\cos\phi + 4.8\cos 2\phi$.

for the divergent values of coupling constants observed between nuclei in nominally *syn*-clinal orientation, as noted above for compounds 17, 18 and 19. In the case of compound 17, the 1,3-dioxane ring is anticipated to be flattened (107). Such flattening, which is a normal feature of 6-membered rings, will have the effect of increasing *syn*-clinal torsion angles above 60° and decreasing the *anti*-periplanar torsion angle below 180°. Using the Karplus-type equation derived above, the torsional angles of 17 can be calculated to be 77° for the *syn*-clinal angle and 147° for the *anti*-periplanar angle, however, considering the 1,3-dioxane structure and the axial methyl group at C-5, such extensive flattening does not seem tenable. The cause of the low observed values for both coupling constants in 17 seems most likely to be the strongly electronegative substituent on the C-2 nucleus which is expected (34) to have an appreciable diminishing influence upon the coupling of C-2 to the vicinal protons.

With regard to compounds 18 and 19, the non-bonded interaction between the methylene group and the methyl group must be substantially greater than that between the methyl group and H-1, when both torsion angles are 60°. It would thus be expected that the CH_3/H angle will be reduced, and the CH_3/CH_2 angle increased, to relieve the steric interactions somewhat. Using the above-derived Karplus-type expression, torsion angles of about 53° and 67° respectively, are estimated. These angles are clearly within reasonable limits of expectation and thus provide satisfactory explanations of the observed coupling constants. Rader (46), working in the related H-C-O-H coupling system, has observed similar increases in coupling constant and proposed a similar explanation, based upon decreased torsion angles, for his observations. Thus, as a result of these model investigations, it is considered that a useful Karplus-type relationship was established for the coupling system, $^{13}CH_3$-O-C-H, under consideration. This relationship will now be applied to the assessment of data obtained in the n.m.r. studies of methyl glycopyranosides.

B. Syntheses of ^{13}C-Labelled Alkyl Glycopyranosides

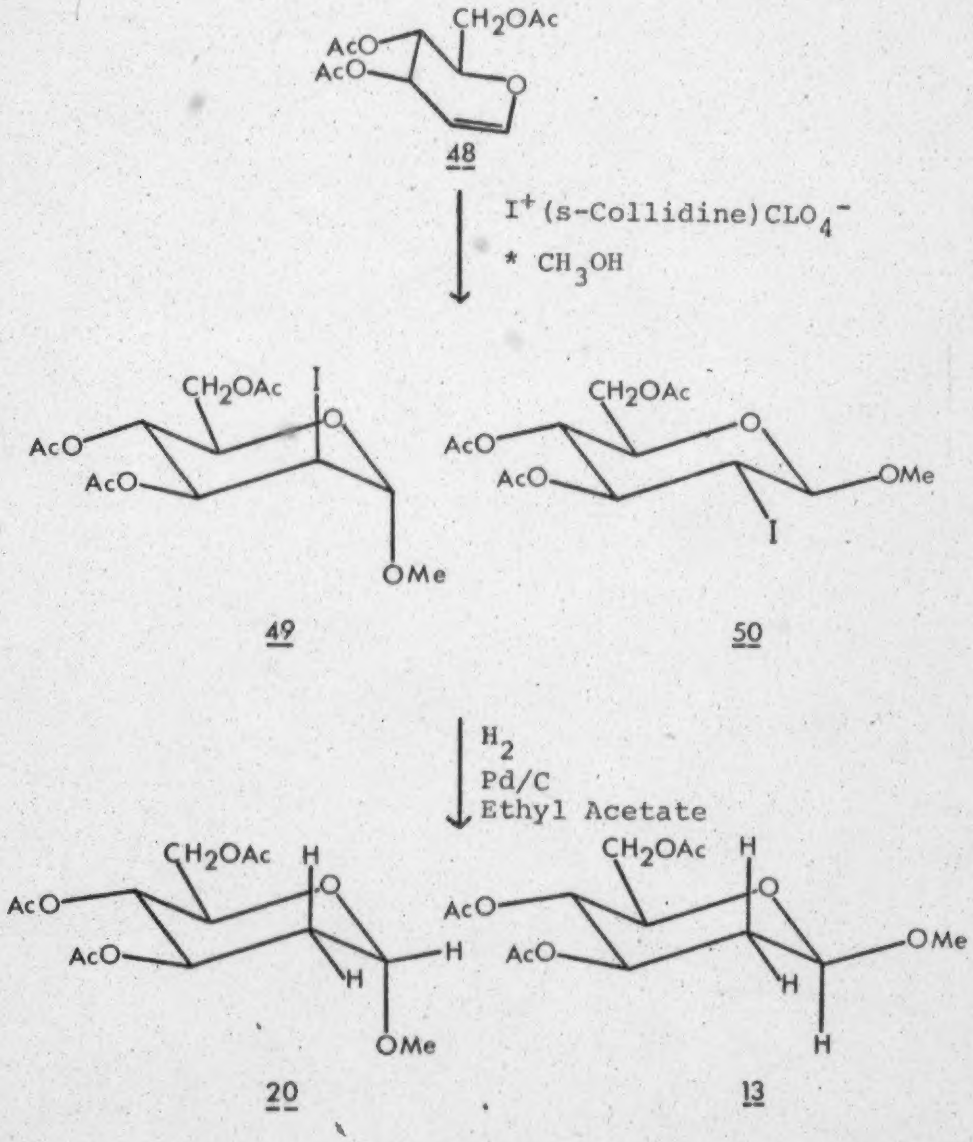
For the synthesis of the alkyl-^{13}C glycosides a major factor that was required to be considered was economy in the use of the enriched methanol reagent. For this reason, a variety of efficient synthetic methods were selected or developed from available literature procedures.

1. Methyl-^{13}C Glycopyranosides

(a) 2-Deoxy-D-*arabino*-hexopyranosides

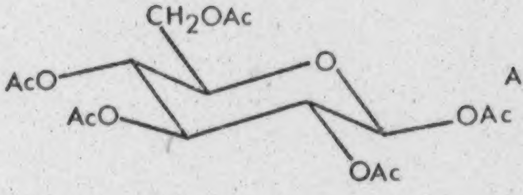
The methyl-^{13}C 2-deoxy-α and -β-D-*arabino*-hexopyranosides were synthesized by the method of Lemieux and Morgan

(86) as shown below. A slight excess of methanol-^{13}C was reacted with D-glucal triacetate (48),under iodo-methoxylation conditions. The resulting 2-deoxy-2-iodo-D-manno- (49) and -glucopyranosides (50) were then reacted with hydrogen in the presence of palladium catalyst to replace the iodo-substituent by hydrogenolysis. The anomeric mixture was separated into its components 20, and 13, by column chromatography.

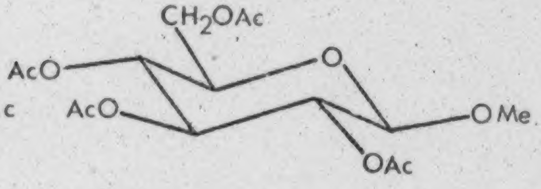

CH2OAc
AcO
AcO
O
48
I+(s-Collidine)CLO4−
* CH3OH
CH2OAc
I
AcO
AcO
O
OMe
49
CH2OAc
AcO
AcO
O
OMe
I
50
H2
Pd/C
Ethyl Acetate
CH2OAc
H
AcO
AcO
O
H
H
OMe
20
CH2OAc
H
AcO
AcO
O
OMe
H
H
13

(b) D-Glucopyranosides

In the case of the methyl-^{13}C glucosides, carbon-13 enriched methanol in slight excess was reacted with penta-*O*-acetyl-β-D-glucopyranose, (51) in the presence of a catalytic quantity of stannic chloride (89). The resulting methyl-^{13}C tetra-*O*-acetyl-β-D-glucoside (23) was then used as starting material for the synthesis of the α-anomer (24) by treatment with titanium tetrachloride to effect anomerization (91).


CH2OAc
AcO
AcO
OAc
OAc
O

51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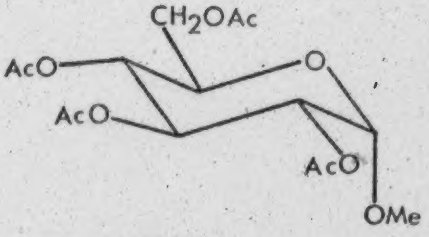
AcO
AcO
OMe
OAc
O

23

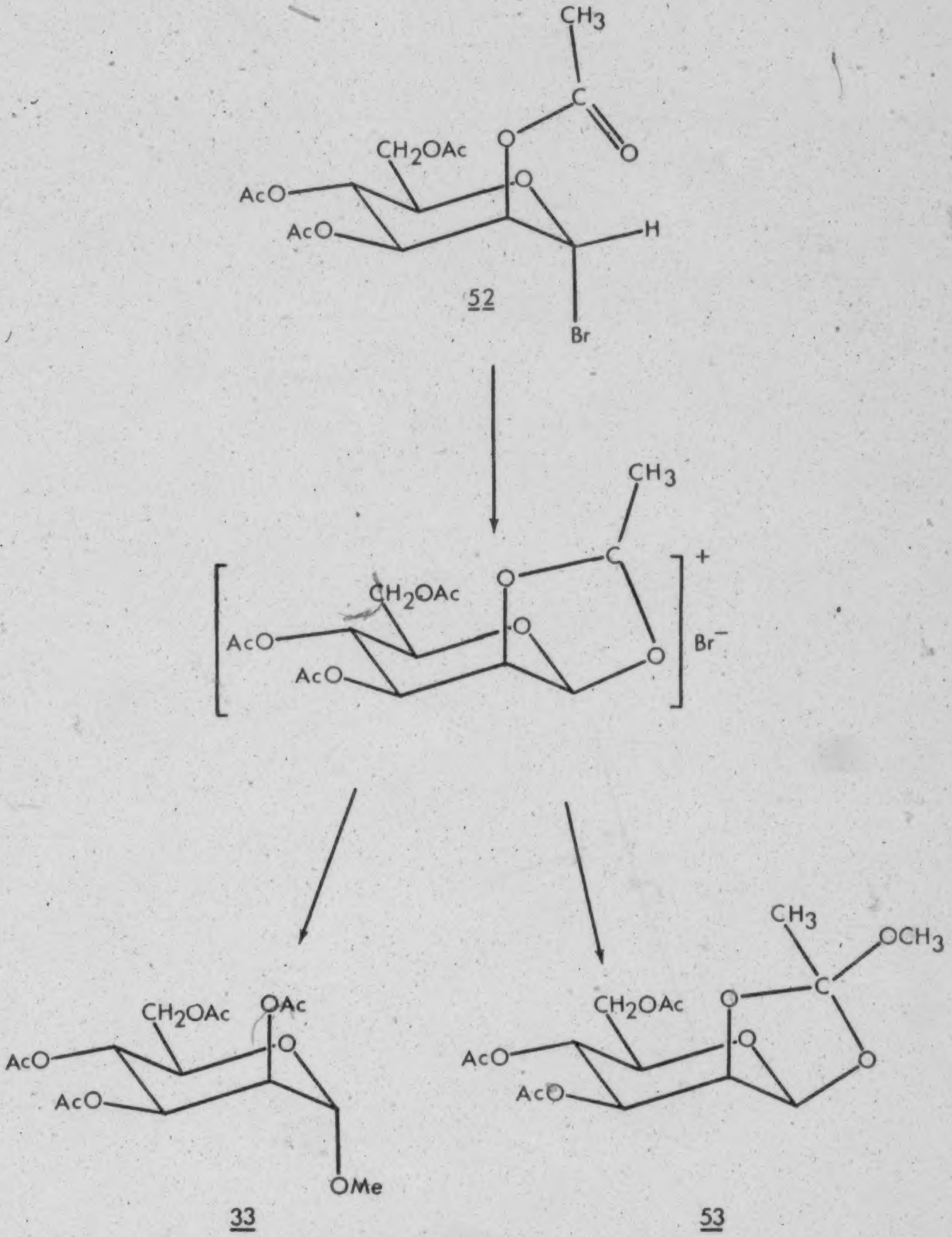

CH2OAc
AcO
AcO
AcO
OMe
O

24

(c) D-Mannopyranosides

The synthesis of methyl-^{13}C β-D-mannoside, in high yield, and with efficient use of methanol-^{13}C, presented a special problem and a suitable procedure could not be found in the literature. The chief factor which operates unfavourably when reactions, normally successful in the glucose series, are attempted in the mannose series, is participation by the axial substituent at C-2 in reactions at the anomeric center. Thus, when mannose is suitably protected and activated for Koenigs Knorr-type glycosidation, by conversion to 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (52), the acetyl substituent at C-2 and the bromide substituent at C-1 have the *anti*-periplanar relationship. This orientation is well known (108) to favour anchimeric participation as illustrated below. The resulting acetoxonium ion species may condense with the methanol reagent to form either the 1,2-orthoacetate 53, or the α-glycoside 33, but not the β-glycoside.

Perlin (109) has successfully overcome this difficulty by utilization of a 2,3-carbonate ester derivative in order to prevent participation by the C-2 substituent. However, this synthesis involves a lengthy series of intermediates with low overall yield and it was therefore decided to adapt a method recently developed for glucoside synthesis (9). This method utilizes a non-participating


CH3
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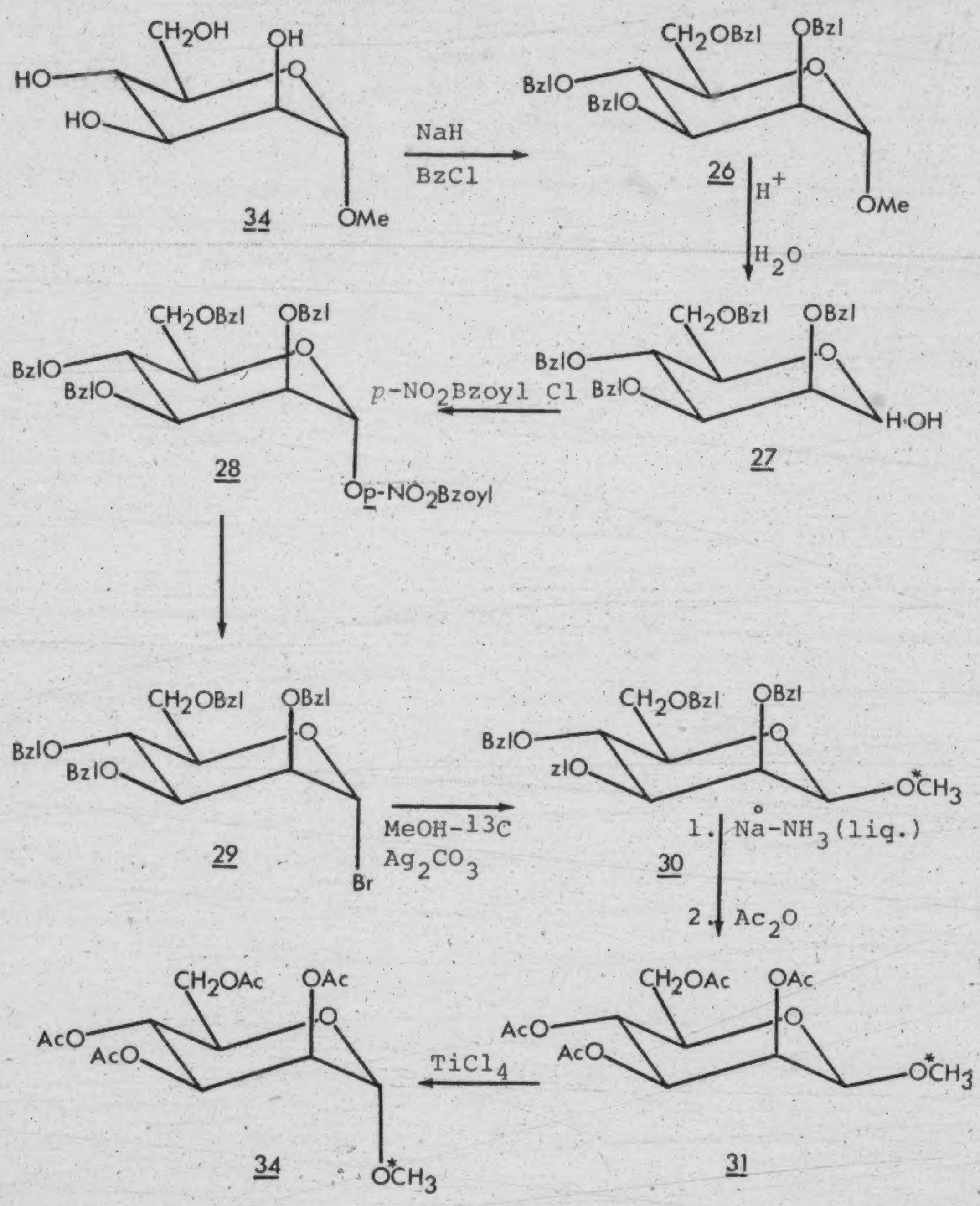
AcO
AcO
H
Br
52
CH3
CH2OAc
AcO
AcO
+
Br−
CH3
OCH3
CH2OAc
OAc
AcO
AcO
OMe
33
CH2OAc
AcO
AcO
53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OH
HO
HO
OMe
34
NaH
BzCl
CH2OBzl
OBzl
BzlO
BzlO
OMe
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H2O
CH2OBzl
OBzl
BzlO
BzlO
H·OH
27
p-NO2Bzoyl Cl
CH2OBzl
OBzl
BzlO
BzlO
Op-NO2Bzoyl
28
CH2OBzl
OBzl
BzlO
BzlO
Br
29
MeOH-13C
Ag2CO3
CH2OBzl
OBzl
BzlO
zlO
O*CH3
30
1. Na-NH3(liq.)
2. Ac2O
CH2OAc
OAc
AcO
AcO
O*CH3
31
TiCl4
CH2OAc
OAc
AcO
AcO
O*CH3
34

benzyloxy substituent at C-2. Thus, methyl α-D-mannopyranoside (34) was converted to its tetra-*O*-benzyl derivative, 26, by reaction with sodium hydride and benzyl chloride in hexamethyl phosphoric triamide. The resulting product was subjected to acid hydrolysis to give 2,3,4,6-tetra-*O*-benzyl-D-mannose (27) which was esterified in pyridine with *p*-nitrobenzoyl chloride to give 28. Compound 28, in turn, was reacted with hydrogen bromide to provide tetra-*O*-benzyl-α-D-mannopyranosyl bromide, 29, and this material proved suitable for reaction with the enriched methanol. The benzyl groups were removed from 30, under Birch reduction conditions, using a literature procedure (95). The methyl-^{13}C tetra-*O*-acetyl-β-D-mannoside, obtained on work up of the hydrogenolysis, was then used as the starting material for preparation of its α-anomer by treatment with titanium tetrachloride to effect anomerization.

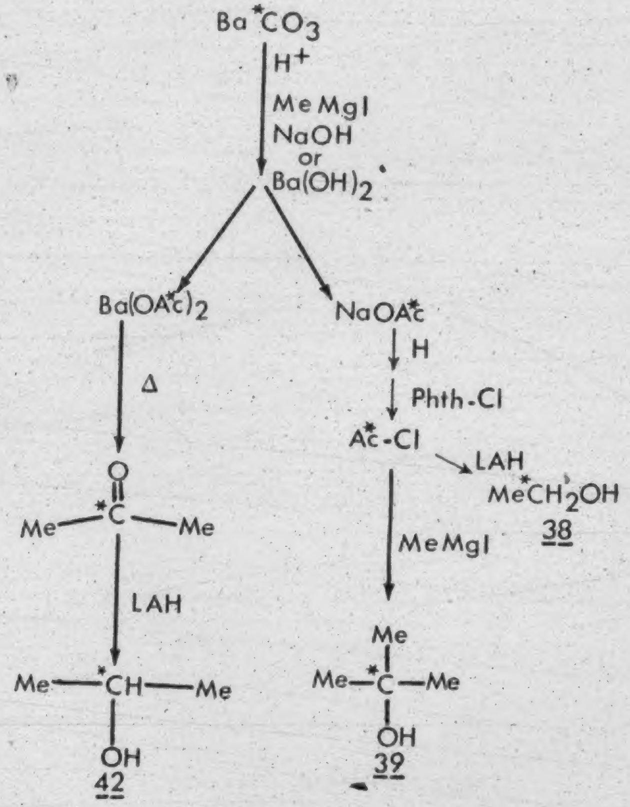
The free methyl-^{13}C mannopyranosides were obtained by deacetylation using standard techniques.

2. Ethyl-1'-^{13}C, Isopropyl-2'-^{13}C, and *t*-Butyl-2'-^{13}C, α- and -β-D-Glucopyranosides

(a) The Carbon-13 Enriched Alcohols

As was the case for the synthesis of the methyl-^{13}C glycopyranosides, a major factor in the selection of suitable synthetic methods leading to the desired alkyl-enriched D-glucosides, was economy in the use of carbon-13 reagents.

In this regard, it is important that adequate precautions be taken to protect the purity and dryness of the carbon-13 enriched alcohols if efficient glycosidation is to be achieved. To this end, the scheme of reactions shown below was chosen for the synthesis of the required alcohols. These reactions were chosen because of their common intermediates and the reported purity and dryness of the products.


Ba*CO3
H+
MeMgI
NaOH
or
Ba(OH)2
Ba(OA*c)2
NaOA*c
H
Phth-Cl
A*c-Cl
LAH
Me*CH2OH
38
Δ
MeMgI
O
Me
*C
Me
LAH
Me
*CH
Me
OH
42
Me
Me
*C
Me
OH
39

The syntheses of all three alcohols was begun with the reaction of methyl magnesium iodide with carbon-13 enriched carbon dioxide liberated on treatment of barium carbonate with acid (98). For the synthesis of ethanol-1-^{13}C, sodium acetate, obtained on work up of the Grignard reaction, was reacted with gaseous hydrogen chloride, to yield acetic acid-1-^{13}C (99). This material in turn was treated with phthaloyl chloride (99) to form acetyl-1-^{13}C chloride which was reduced with lithium aluminum hydride (99) in the high boiling ether, bis-(2-ethoxyethyl)ether. The purified ethanol-1-^{13}C was stored in a sealed tube until required.

For the synthesis of isopropanol-2-^{13}C, the initial Grignard reaction was worked up by steam distillation of the acidified product and titration of the aqueous acetic acid with solid barium hydroxide. After freeze-drying, solid barium acetate was obtained which was further dried by heating *in vacuo* to 150° C. Acetone-2-^{13}C was then synthesized by pyrolysis of the anhydrous barium acetate *in vacuo* at approximately 500° C. This material was reduced essentially as described for the synthesis of ethanol. The purified isopropanol-2-^{13}C was stored until use in a vacuum trap.

In the case of *t*-butanol-2-^{13}C, acetyl-1-^{13}C chloride was reacted with excess methyl magnesium iodide and the aqueous and ether layers, obtained upon acidification, were transferred to a continuous extraction apparatus. After lengthy extraction of the aqueous layer, the ether was largely separated from the *t*-butanol-2-^{13}C by distillation and the ether/*t*-butanol mixture so obtained was shaken with Linde molecular sieves to remove any remaining water.

(b). Glycosidation and Anomerization

The ethyl-1'-^{13}C, isopropyl-2'-^{13}C, and *t*-butyl-2'-^{13}C tetra-*O*-acetyl-β-D-glucopyranosides were synthesized, in yields approaching 60% by the reaction of the enriched alcohols, described above, with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric bromide and mercuric oxide (110). In the case of the ethyl and isopropyl compounds, the α-anomers were synthesized from their respective alkyl tetra-acetyl β-anomers, by treatment with titanium tetrachloride, as already described for the methyl glucosides.

In all cases the free alkyl glucosides were obtained by deacetylation using standard methods.

C. The Conformational Properties of the *cis* and *trans* Isomers of 1-Methoxy-4-*t*-butylcyclohexane (18 and 19)

Compounds 18 and 19 were prepared, enriched with carbon-13 in the methyl group, as was previously described.

Because of the kinetic effect of temperature upon internal rotation (2) it was of interest to determine the vicinal coupling constants between the ^{13}C nucleus and H-1 over a broad range of temperatures. The data obtained for both compounds are presented in Fig. 18. A decrease is observed in the coupling constants of both compounds, with decreasing temperature. This decrease is expected to result from depletion of the population of the *anti*-periplanar rotamers, 18c and 19c, which are the least stable of the three staggered conformers of the methyl group.

The coupling constants are seen both to become equal to 3.35 Hz at 220° K. Thus, it is apparent that the contributions of the *anti*-periplanar forms to the observed coupling constants becomes negligible at this low temperature. The value observed, 3.35 Hz, therefore is expected to reflect the actual coupling constant for the methyl-^{13}C to H-1 coupling when these nuclei are in *syn*-clinal relative orientation. As was noted previously, the methyl group may be assumed to adopt a torsion angle of somewhat less than 60°, relative to H-1, because of the difference in steric bulk between the methylene group on one side and H-1 on the other. On the basis of the Karplus-type relationship presented in Fig. 18, the *syn*-clinal torsion angle is determined to be 55°, which is reasonable in light of the above discussion.

The value of 3.35 Hz for coupling between *syn*-clinal nuclei together with the value of 10.6 Hz obtained

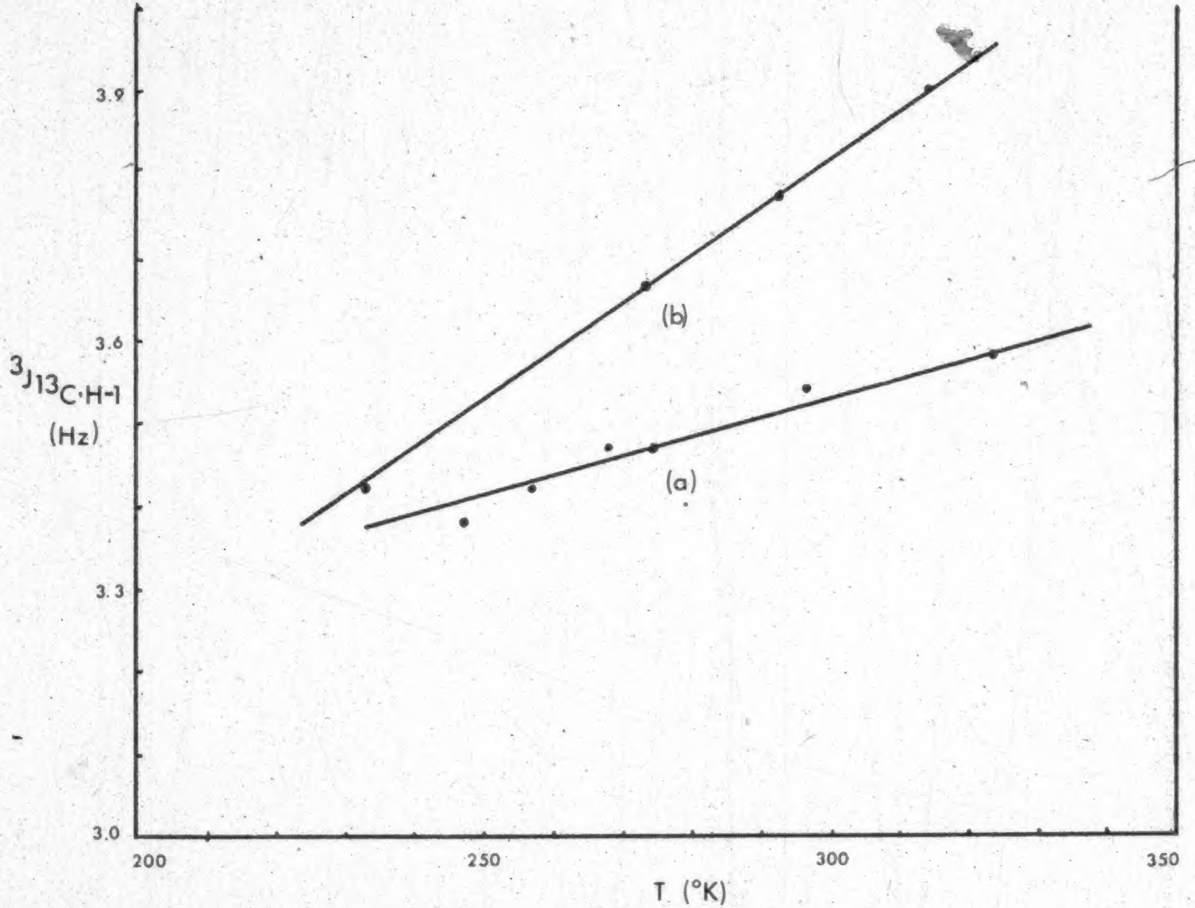


Fig. 19: Graph of the temperature dependence of $^{3}J_{^{13}C,H\text{-}1}$ for a) *cis*-1-methoxy-4-*t*-butylcyclohexane (18) and b) the *trans*-isomer (19).

from Fig. 17, for coupling between nuclei in *anti*-periplanar orientation will now be used to estimate the conformational equilibria for these compounds at 30° C (303° K), which is the normal operating temperature of the n.m.r. probe used in this research.

The coupling constant for *cis*-1-methoxy-4-*t*-butylcyclohexane (18) is estimated from the plot in Fig. 18 to be 3.53 Hz. Therefore, where n is the total mole fraction of the *syn*-rotamers, 18a and 18b, the equation,

$$J = 3.35\ n + 10.6\ (1-n) = 3.53,$$

may be solved, and n is found to be 0.975. It is concluded therefore, that the conformational equilibrium is approximately,

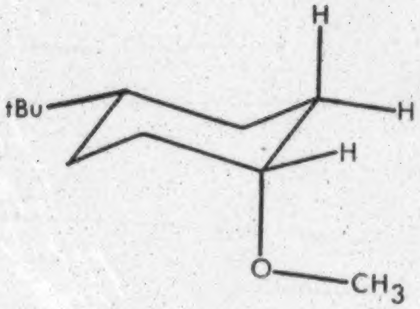
$$n_{\underline{18a}} = n_{\underline{18b}} = 0.488, \text{ and}$$

$$n_{\underline{18c}} = 0.024.$$

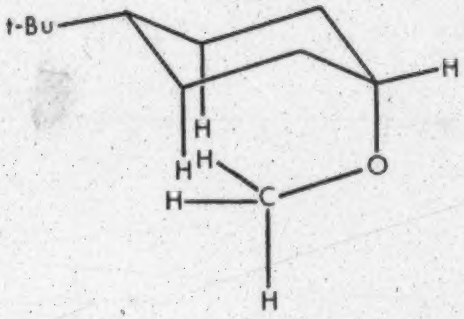
On this basis, the difference in free energy ($\Delta G°$) between either 18a or 18b and 18c is estimated from the expression,

$$\begin{aligned} -\Delta G° &= RT\ln K_{eq}, \\ &= 2 \times 303 \times \ln \frac{0.488}{0.024}, \\ &= 1.83 \text{ kcal/mole.} \end{aligned}$$

An appreciation of the origin of this large difference in stability can be gained from a detailed conformational analysis, as follows.



18a or 18b



18c

The conformational analysis (13) for 18c is:

$$2CH_3//CH + 2e//H + 2e/H.$$

For compounds 18a or 18b the analysis is:

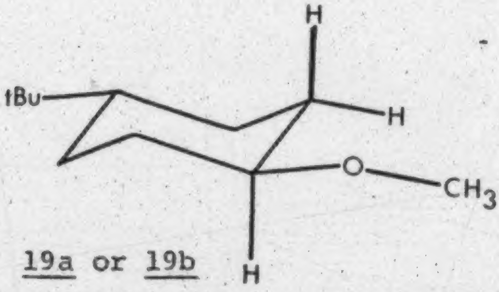
$$CH_3//H + 2e//CH + e//H + CH_3/H + e/H.$$

Therefore, the difference in non-bonded interactions between 18c and either 18a or 18b is:

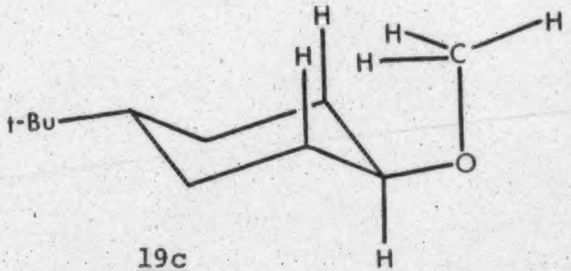
$$2(CH_3//CH - e//CH) - (CH_3//H - e//H)$$

$-(CH_3/H - e/H)$, which was calculated above to be 1.83 kcal/mole.

This value is actually small in comparison to those found in standard texts, but it may be noted that a proper explanation will have a nuclear-electron attractive component when unshared pairs of electrons are involved (27).



19a or 19b



19c

For 19c, the analysis is:

$$2CH_3//H + 2e//H + 2e/H,$$

while for 19a or 19b, the analysis is:

$$CH_3//H + 3e//H + CH_3/H.$$

The difference in non-bonded interactions between 19c and either 19a or 19b is:

$$(CH_3//H - e//H) - (CH_3/H - e/H),$$

which is found to be 1.21 kcal/mole.

This value for the non-bonded interaction free energy difference was calculated using the coupling constant, 3.83 Hz, obtained from the graph for 19 in Fig. 19. Thus, with n representing the total mole fraction of 19a and 19b, the following equation was solved for this quantity:

$$J_{obs} = 3.35\ n + 10.6\ (1-n) = 3.83.$$

From the result obtained, the conformational equilibrium at 303° K for 19 is approximately,

$$n_{\underline{19a}} = n_{\underline{19b}} = 0.468, \text{ and}$$

$$n_{\underline{19c}} = 0.064.$$

The equilibrium constant is thus 7.3 in favour of 19a or 19b, which corresponds to the free energy difference of 1.21 kcal/mole reported above.

In passing, it is of interest to note that the expression for 19c - 19a can be written as

$$(CH_3//H - CH_3/H) - (e//H - e/H) = 1.21 \text{ kcal/mole.}$$

Studies (111) of the conformational equilibrium of methylcyclohexane provide a value of 0.85 kcal/mole for the nonbonded interaction free energy difference, $CH_3//H - CH_3/H$. Thus, it is apparent that, either the shorter C-O bond results in increased interaction in 19, while the term (e//H - e/H) is very small, or, alternatively, the latter term has a negative value and attraction, rather than repulsion, exists between unshared electron pairs and an interacting hydrogen atom.

The purpose of the above-described study will become evident when the conformational analyses of methyl glycopyranosides are discussed in the following section.

D. The Conformational Properties of Methyl Glycopyranosides

1. Indications of Conformational Preference - Optical Rotation Studies

Table 5 lists the molecular rotation constants for a number of glycopyranoses and their methyl glycopyranosides. It is seen that the magnitude of the difference in rotation

TABLE 5

The Molecular Rotation Constants of Some D-Glycopyranoses and Their Methyl Glycopyranosides

Compound	$[M]_D$ (H_2O)††	Δ $[M]_D$ (OCH_3)†
2-Deoxy-α-D-*arabino*-hexopyranose	130°*	+117°
Methyl 2-deoxy-α-D-*arabino*-hexopyranoside	247	
α-D-Glucopyranose	202	+107
Methyl α-D-glucopyranoside	309	
α-D-Mannopyranose	53	+104
Methyl α-D-mannopyranoside	157	
α-D-Galactopyranose	272	+108
Methyl α-D-galactopyranoside	380	
2-Deoxy-β-D-*arabino*-hexopyranose	30*	-116
Methyl 2-deoxy-β-D-*arabino*-hexopyranoside	- 86	
β-D-Glucopyranose	34	-100
Methyl β-D-glucopyranoside	- 66	
β-D-Mannopyranose	- 31	-104
Methyl β-D-mannopyranoside	-135	
β-D-Galactopyranose	+ 95	- 95
Methyl β-D-galactopyranoside	0	

* Values calculated by Brewster (74)

† $[M]_D$ (glycoside) - $[M]_D$ (glycose)

†† Values taken from Whiffen (73) and Brewster (74).

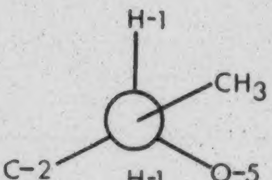
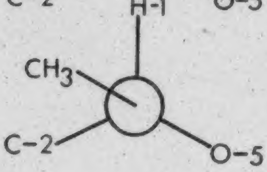
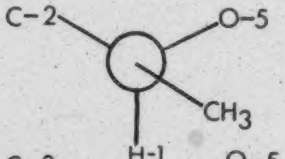
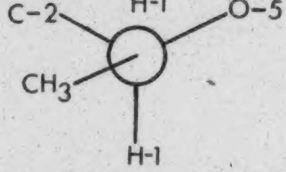
Rotamer	Structure	Asymmetric* Unit	Contribution*
α-a		$+C_o/O$	+115°
α-b		$-C_o/C$	- 65°
β-a		$-C_o/O$	-115°
β-b		$+C_o/C$	+ 65°

Fig. 20: The asymmetric units of some glycosidic bond rotamers, and their contributions to molecular optical rotation in methyl D-glycopyranosides.

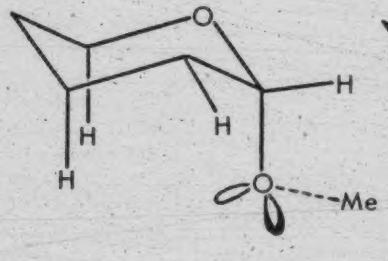
*Lemieux and Brewer (76).

which arises upon introduction of the methyl group is remarkably large and constant for a given pair of compounds of the same configuration. It seems appropriate to discuss the values observed in terms of the three-bond units of asymmetry formed by the rotamers about the glycosidic bond. The α-c and β-c forms have the contributing atoms in *anti*-periplanar orientation and therefore make no contribution to the molecular rotation but the other rotamers are all expected to be optically active, as seen in Fig. 20. The values assigned to the contributions are of course only approximate and other authors (73,74) have used slightly different values. However, it is evident that the observed contributions of the methyl group found in Table 5, are indicative of virtually exclusive populations of the α-a and β-a rotamers only. It is clear that the methyl group is in the same environment regardless of the absence, or presence, of an hydroxyl group at C-2 in axial or equatorial configuration. A C-2 hydroxyl group in equatorial orientation should disfavour the α-b and β-b conformers, while a C-2 axial hydroxyl should disfavour the β-c conformer. However, no important variations in molecular rotation can be found which would indicate that any of these changes occur where expected. Thus, the conclusion that the α-a and β-a rotamers predominate, is supported.

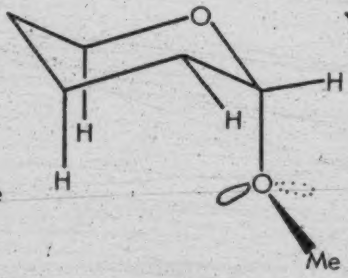
2. Investigations into Conformational Preference

(a) Conformational Analyses and Temperature Studies - $^{3}J_{C,H}$

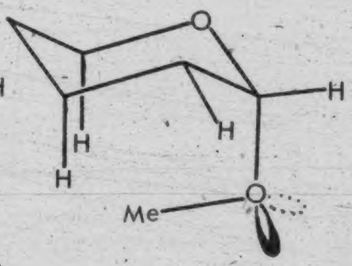
A discussion of the conformational properties of methyl glycopyranosides which result from rotation about the C-1 to O-1 bond, seems best approached by conformational analyses based upon consideration of the staggered conformers. For the purposes of this discussion the terms αa, αb and αc are intended to refer to the non-bonded interactions which occur in the α-a, α-b and α-c type rotamers, respectively, of a methyl-2-deoxy-α-D-glycopyranoside.



α-a



α-b



α-c

Thus,

$\alpha a = e//CH_3 + 2e//CH + e//H + CH_3/H + e/H$,

$\alpha b = CH_3//H + 2e//CH + e//e + CH_3/H + e/H$, and

$\alpha c = 2CH_3//CH + e//e + e//H + 2e/H$.

It is evident at once that the energies of all of these conformers are different. It has been pointed out (112) that the occurence of the e//e interaction term is useful to identify the anomeric (or *exo*-anomeric) effect, but its use in this context does not necessarily refer to the physical origin of these effects. It is seen from these analyses that this strongly destabilizing influence is found in the αb and αc conformers, but not in αa. The αa conformer is also more stable because of the relatively small non-bonded interactions present in it. This latter factor is readily appreciated by subtracting the non-bonded interaction equations for the α-a from those of the α-b and α-c conformers.

Consider first the α-a and α-c rotamers:

$$\alpha c - \alpha a = 2(CH_3//CH - e//H) - (e//CH_3 - e//e) - (CH_3/H - e/H).$$

In the case of the *cis*-ether, 18, it was found experimentally that

$$2(CH_3//CH - e//CH) - (CH_3//H - e//H) - (CH_3/H - e/H)$$

$$= 1.83 \text{ kcal/mole}$$

$$\text{or} \quad 2(CH_3//CH - e//CH) - (CH_3/H - e/H)$$

$$= 1.83 + (CH_3//H - e//H).$$

When this expression is substituted into the equation $\alpha c - \alpha a$, then

$$\alpha c - \alpha a = 1.83 + (CH_3//H + e//e) - (e//CH_3 + e//H).$$

The terms CH_3//H and e//e are, without question, large and positive. Also, as was indicated above, terms such as e//CH_3 and e//H are either small or negative. In the latter case the conformational driving force in favour of α-a would be exceptionally large.

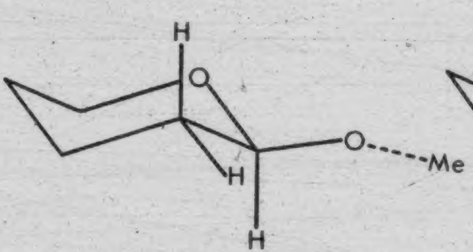
When the α-a and α-b type rotamers are considered,

$$\alpha b - \alpha a = (CH_3//H + e//e) - (e//CH_3 + e//H).$$

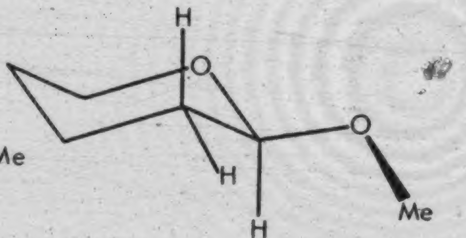
It is evident, for the same reasons as outlined above in consideration of αc and αa, that the α-a conformer must be expected to be much more favoured than the α-b form.

The indications are, therefore, that 2-deoxy-α-glycopyranosides should exist extensively in the α-a type conformer. This natural tendency should be further reinforced by the presence of bulky equatorial substituents at C-2 of the pyranoid ring, as in the glucosides.

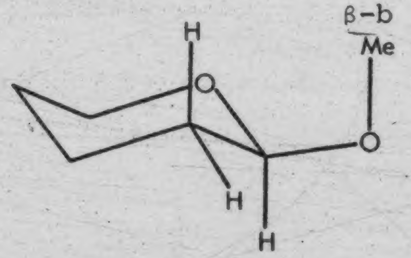
In the case of a methyl 2-deoxy-β-D-glycopyranoside, the conformational analysis is as follows:



β-a



β-b



β-c

$\beta a = e//CH_3 + 2e//H + e//e + CH_3/H + e/H$,

$\beta b = CH_3//H + e//H + 2e//e + CH_3/H + e/H$, and

$\beta c = CH_3//H + e//CH_3 + e//e + e//H + 2e/H$, where βa, βb and βc are defined analogously to αa, αb and αc. By following the procedure used above in the analysis of the 2-deoxy-α-glycopyranoside, it is seen that,

$$\beta c - \beta a = (CH_3//H - e//H) - (CH_3/H - e/H).$$

This is the same non-bonded interaction difference as that derived from the analysis of the *trans*-ether 19, which was found experimentally to be equal to 1.21 kcal/mole. Thus, evidently the β-a conformer is substantially more stable than the β-c form. However, with a non-bonded interaction free energy difference of only 1.21 kcal/mole, β-c can be expected to have a population of about 0.064 mole fraction. In order to examine this possibility, the ^{13}C to H-1 coupling was determined for methyl-^{13}C tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside in DMSO-d6 over the temperature range 32° C to 72° C. No variation in the coupling constant, 4.3 ± 0.1 Hz was observed. From this observation, it is apparent that the β-c conformer has a mole fraction substantially less than 0.06. This matter was further investigated using methyl-^{13}C β-D-glucopyranoside and the results are presented in Figs. 21 and 22. It is seen that, in either D_2O or DMSO solution, only a slight increase in the coupling constant, $^3J_{C,H-1}$ was observed. It is not possible to determine the coupling constant for these compounds in conformation β-a.

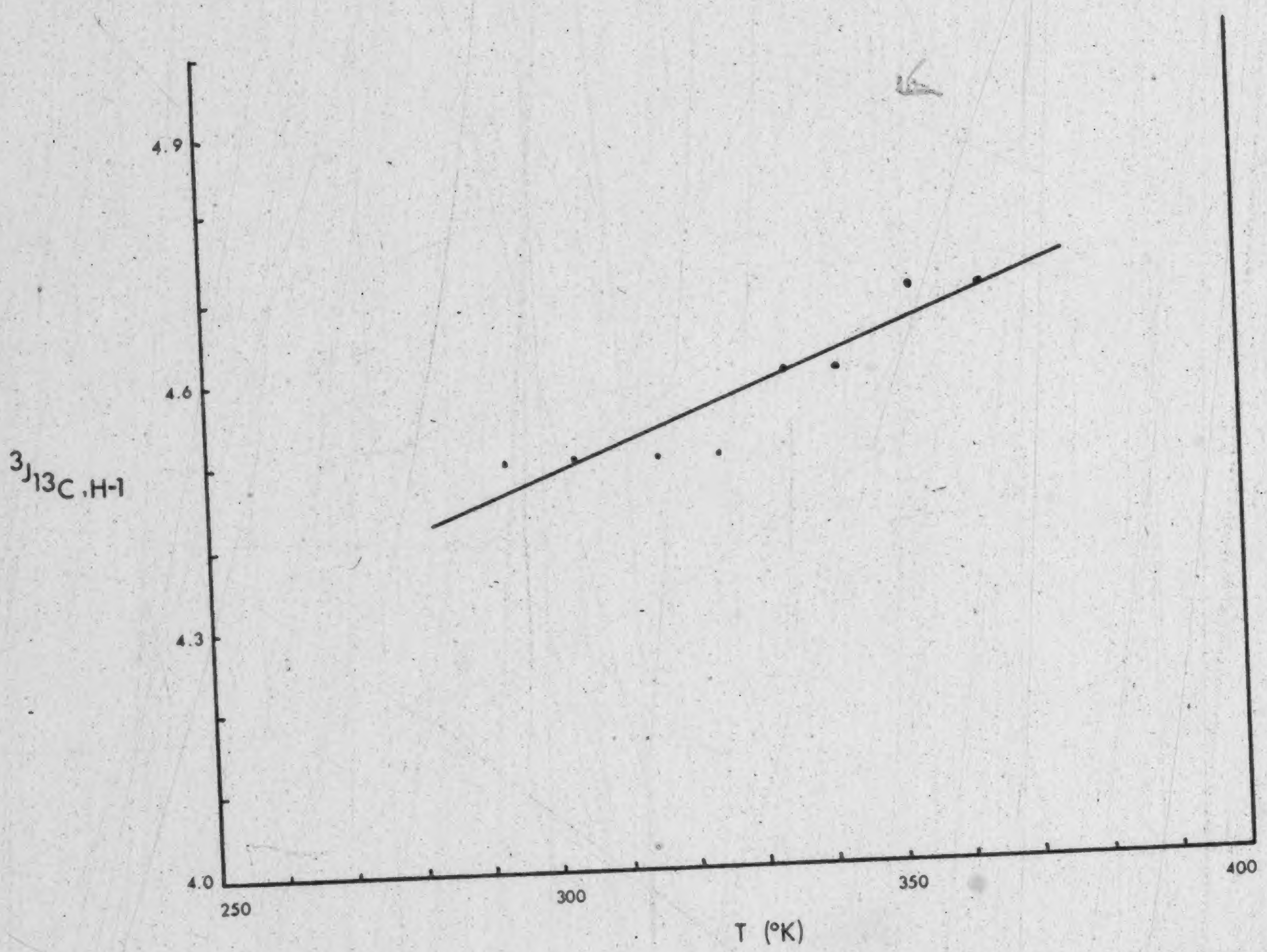


Fig. 21: The effect of temperature on the vicinal coupling between aglyconic carbon and anomeric proton in methyl-^{13}C β-D-glucoside dissolved in D_2O.

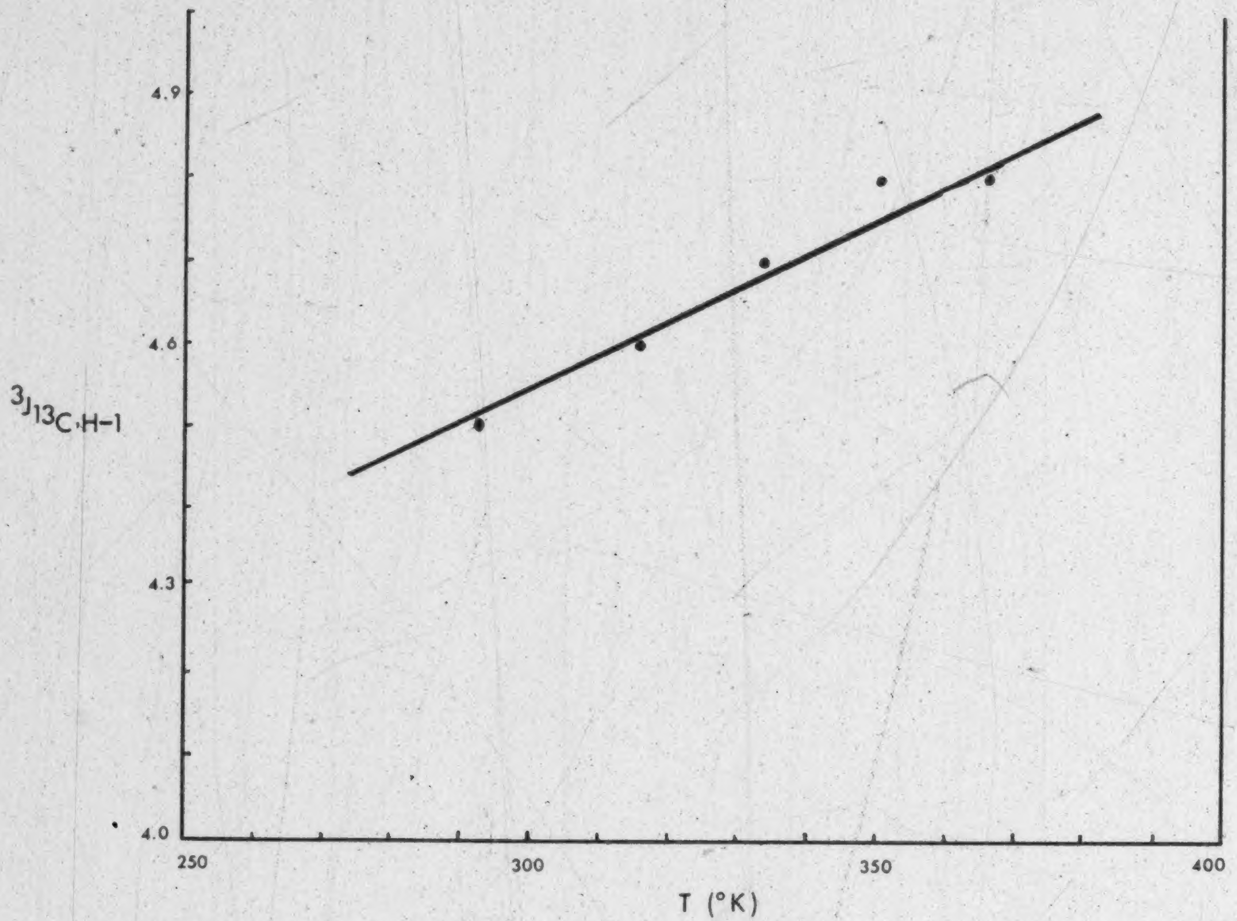


Fig. 22: The variation of $^3J_{^{13}C,H-1}$ with temperature for methyl-^{13}C β-D-glucoside in DMSO-d6.

However, if it is assumed that the value of this constant is not less than 4.3 Hz, then it may be inferred that the mole fraction of the β-c form is not greater than 0.03, by the use of calculations similar to those performed for the ethers 18 and 19.

For the β-b and β-a rotamers the conformational analysis provides

$$\beta b - \beta a = (CH_3//H + e//e) - (e/CH_3 - e//H).$$

It follows, therefore, from arguments similar to those presented above, that no appreciable amount of β-b can be expected to exist for β-glycopyranosides. In the case of glucosides, in which a $CH_3//O$ non-bonded interaction replaces the $CH_3//H$ interaction, this analysis is reinforced still further.

The above conclusions, based on conformational analysis, that conformations other than the a-type have negligible populations, are supported by a number of other experimental data.

(b) Consideration of Other Coupling Constant Data

The vicinal aglyconic carbon to anomeric hydrogen coupling constants of the free methyl-^{13}C glycosides were determined in water, methanol and DMSO, with the results shown in Table 6.

TABLE 6

Carbon-13 N.M.R. Spectral Data for Some Methyl-^{13}C D-Glycopyranosides in Water, DMSO and Methanol

Compound	Solvent*	$^3J_{^{13}C\text{-O-C-H}}$	$^1J_{^{13}C\text{-H}}$	δ_{TMS}
2-deoxy-α-D-*arabino*	Methanol	3.2	145	—
" " "	DMSO	3.2	141.8	54.8
" " "	Water	3.2	141.8	54.4†
α-D-*manno*	Methanol	3.8	142	55.3
"	DMSO	3.7	143.5	—
"	Water	3.8	144.1	—
α-D-*gluco*	Methanol	3.9	143.3	—
"	DMSO	3.5	142.1	55.3
"	Water	3.8	144.6	—
2-deoxy-β-D-*arabino*	Methanol	4.4	—	—
" " "	DMSO	4.3	142.3	—
" " "	Water	4.2	145.1	—
β-D-*manno*	Methanol	4.2	142.9	57.1
"	DMSO	4.2	142.3	57.4††
"	Water	4.3	144.1	56.8†
β-D-*gluco*	Methanol	4.5	143.0	—
"	DMSO	4.5	143.0	—
"	Water	4.5	144.5	—

* Deuterated solvents were used.

† Calculated by adding 30.2 p.p.m. to the chemical shift measured from the methyl carbons of acetone.

†† Calculated by adding 41.1 p.p.m. to the chemical shift measured from the methyl carbons of DMSO.

When the data for the α-anomers are examined it is seen that the coupling constants found for all three pyranosides are consistent with *syn*-clinal orientation of the methyl group with respect to H-1. Moreover, even in solvents of widely differing characteristics there is very little change in the values observed for any compound. Thus, solvents of high dielectric constant, ε, such as water, may be expected to facilitate occupancy of the α-c rotamer, which has an unfavourable orientation of bond dipoles. However, the increase in coupling constant which would be expected to result in water ($\varepsilon = 80$) compared to methanol ($\varepsilon = 33$), is not observed. It is indicated, therefore, that the relatively higher values found for the glucoside and mannoside do not arise from increased occupancy of the α-c rotamer in these compounds, when compared to the 2-deoxy-α-D-*arabino*-compound. It is evident from these results that the variations, which occur between the different α-pyranosides, are the result of subtle changes in the torsion angle of the α-a rotamer, rather than in redistributions of the relative rotamer populations.

In the case of the β-anomers, somewhat larger coupling constants are observed compared to those found in their α-anomers. An obvious source of this larger value may appear to lie in possible increased populations of the β-c rotamer, which may be expected to contribute strongly to this coupling. However, the continued high value found for

TABLE 7

Carbon-13 N.M.R. Spectral Data for Some Methyl Per-*O*-acetyl-D-glycopyranosides

Compound	Solvent*	$^{3}J_{^{13}\text{C-O-C-H}}$†	$^{3}J_{^{13}\text{C-H}}$†	δ_{TMS}††
2-deoxy-α-D-*arabino*	$CDCl_3$	3.4	143.8	55.5
" " "	Acetone	3.3	142	54.7
" " "	DMSO	3.7	142.6	54.3
α-D-*manno*	$CDCl_3$	3.9	143.1	55.3
"	Acetone	3.7	143.1	55.3
"	DMSO	3.6	143.5	54.3
α-D-*gluco*	$CDCl_3$	3.6	143	55.8
"	Acetone	3.1	—	55.7
"	DMSO	3.2	142.9	56.3
2-deoxy-β-D-*arabino*	$CDCl_3$	4.3	—	57.0
" " "	Acetone	4.2	142.8	—
" " "	DMSO	4.2	143	57.9
β-D-*manno*	$CDCl_3$	4.4	143.5	57.3
"	Acetone	4.3	143.3	57.0
"	DMSO	4.3	143.3	—
β-D-*gluco*	$CDCl_3$	4.3	143	56.7
"	Acetone	4.2	142	56.5
"	DMSO	4.2	143.6	57.7

* All spectra were measured in perdeuterated solvents.

† Coupling constants are in Hz.

†† Chemical shifts in p.p.m. are from the methyl carbon of TMS used as an internal standard.

$^3J_{C,H}$ in the mannoside, 32, in which the β-c rotamer is significantly less favourable, is strong evidence against this interpretation. Further evidence concerning this phenomenon will be forthcoming later in this thesis. For the present, the higher coupling constants found for the β-anomers are interpreted as resulting from relatively smaller torsion angles within the β-a arc, as compared to the α-a arc.

Similar solvent effect studies have been carried out with the per-*O*-acetyl-derivatives of these glycosides with the results reported in Table 6. It is seen that, except for a slight general decrease in the size of $^3J_{C,H-1}$ the features of the free glycosides are reproduced. Thus, for the reasons argued previously, it is concluded that these compounds also adopt the α-a and β-a conformers predominantly.

(c) Vicinal Carbon-Carbon Nuclear Spin Coupling

While coupling between vicinal carbon nuclei has only recently come under investigation, it seemed possible that conformational information might be derived from observation of the coupling constant between aglyconic carbon and C-2 of the pyranoid moiety. These nuclei have the *anti*-periplanar orientation when the methyl aglycon is in the α-a or β-a rotameric form and, this orientation is known to be especially favourable for nuclear spin coupling in both proton-proton (52), and carbon-proton systems (49). The values determined for the methyl 2-deoxy-D-*arabino*- and glucopyranosides are shown in Table 8.

TABLE 8

Vicinal Carbon-Carbon Coupling in Some Methyl-^{13}C D-Glycopyranosides in Water

Compound	$^{3}J_{C,C-2}$ (Hz)
2-deoxy-α-D-*arabino*-hexopyranoside	3.0
α-D-glucopyranoside	2.4
2-deoxy-β-D-*arabino*-hexopyranoside	3.0
β-D-glucopyranoside	3.1

Although the values observed would be considered small in other coupling systems, it is becoming established (58,60,61) that such small values are generally observed in vicinal carbon coupling systems. For example, the vicinal carbon-proton coupling constant for nuclei in *anti*-periplanar orientation in benzene is 7.4 Hz (42) while the analogous carbon-carbon coupling in toluene is only 3.9 Hz (59). Also, in the torsion angle dependence curve developed by Marshall (62), it was seen that the region of null coupling for carbon-carbon vicinal coupling was comparatively broad and extended below 60° of torsion. Thus, the values observed here, in this context, seem quite large, and are consistent with high populations of the α-a and β-a rotamers. The relatively smaller value observed for methyl-^{13}C α-D-glucopyranoside cannot be explained by consideration of the alternate rotamers but may arise from the near complete overlap of the signal for C-2 with that of C-5.

(d) The Methyl Group to H-1 Torsion Angle in Methyl β-D-Glycosides

In Section (c) of this chapter it was noted that the β-glycosides exhibited significantly greater coupling between the aglyconic carbon and anomeric hydrogen nuclei than do the α-glycosides.

An obvious source of this discrepancy would be the existence of a significant population of the *anti*-periplanar β-c rotamer. As was noted previously, this rotamer would be expected to make a relatively large contribution to the observed coupling constant. On the basis of the conformational analysis of the methyl 2-deoxy-β-D-hexopyranoside, however, the maximum population which might be expected for the β-c rotamer of this compound is 0.03 mole fraction. If a certain latitude is allowed, then it might be supposed that as much as 0.06 mole fraction could exist in this form. However, when this latter population is used to calculate the coupling constant expected for this population distribution then,

$$\begin{aligned} J &= 0.94J_{\alpha} + 0.06\ J^{180}, \text{ or} \\ &= 0.94 \times 3.2 + 0.06 \times 10.6, \\ &= 3.65. \end{aligned}$$

J_α is the vicinal carbon to proton coupling observed in the α-anomer, which is assumed to have *syn*-clinal orientation exclusively, and J^{180} is the coupling constant of carbon and proton in *anti*-periplanar orientation, obtained from the Karplus-type relationship (Fig. 17). Clearly, even with a population of β-c which is twice that which may reasonably be expected, the observed coupling constant cannot be calculated. Thus, the explanation is untenable that the relatively larger coupling constants of β-glycosides result from high population of the *anti*-periplanar rotamer.

A second obvious possible source of this phenomenon is that, for some reason, the effective torsion angle between the coupled nuclei is smaller in β-glycosides than in α-glycosides. As was mentioned previously, the Karplus-type curve for vicinal carbon-proton coupling is very steep in the *syn*-clinal arc, so that only small differences in the torsion angle provide large changes in the coupling expected. In order to test this possibility, nuclear Overhauser technique experiments were carried out with several different methyl D-glycopyranosides. The results of these experiments are reported in Table 9. These experiments were performed by observation of the signal for the anomeric proton and irradiation at the resonance of the methoxyl protons. In this way, the relative spatial proximity of the methyl group and anomeric proton were determined. From the data in Table

TABLE 9

Nuclear Overhauser Enhancements in Some Methyl Glycopyranosides in Water

Compound	N.O.E. (%)*	σ † (%)
α-D-glucoside	0	5
6-deoxy-α-D-glucoside	8	2
α-D-mannoside	8	6
6-deoxy-α-L-mannoside	1	5
β-D-glucoside	12	3
6-deoxy-β-D-glucoside	17	5
6-deoxy-β-L-mannoside	14	2

* These experiments were carried out with purified samples of non-enriched materials. After dissolution and addition of reference compounds the solutions were degassed and sealed in n.m.r. tubes. Experiments with the glucosides were carried out using acetone as the reference n.m.r. lock and dichloromethane as the reference standard of enhancements. Experiments with the mannosides were carried out using pyrazine as the reference n.m.r. lock for technical experimental reasons. Otherwise the conditions were identical. The high r.f. energy required for decoupling resulted in experimental temperatures of 60° ± 5°.

† The standard deviation σ, is defined as the square root of the average of the squared deviations from the mean. A minimum of 8 replicates were used for each experiment.

9, it is evident that significantly greater enhancements are observed for the β-glycosides than for their α-anomers. Thus, it is concluded that the torsion angle between the methyl group and the anomeric proton is smaller in the methyl β-glycosides than is the case for these compounds in the α-configuration.

This conclusion may seem surprising in view of the structural similarity of the α-a and β-a conformers, however, when the conformational analyses of these rotamers are compared, it is seen that they are different. Thus,

$$\alpha a = e//CH_3 + 2e//CH + e//H + CH_3/H + e/H, \text{ and}$$

$$\beta a = e//CH_3 + 2e//H + e//e + CH_3/H + e/H.$$

The difference between these forms is then

$$\alpha a - \beta a = (e//CH - e//e) + (e//CH - e//H).$$

It is noteworthy that only terms for non-bonded interactions involving electrons are present in this expression. It is thus inferred that the electronic structure of the β-glycosidic bond differs materially from that of the α-glycosidic bond. There is evidence which demonstrates this difference in electronic structure. For example, the chemical shifts of the aglyconic carbon of the methyl β-D-glycopyranosides differ significantly from both the α-anomers and the *cis* and *trans* ethers, 18 and 19. The relevant data are presented in Table 10.

TABLE 10

The Carbon-13 Chemical Shifts of the Methoxyl Carbon in Some Methyl Glycopyranosides and Methoxy Cyclohexanes

Compound	Solvent	^{13}C (p.p.m.*)
cis-1-Methoxyl-^{13}C-4-*t*-butylcyclohexane	$CDCl_3$	55.5
trans-1-Methoxyl-^{13}C-4-*t*-butylcyclohexane	$CDCl_3$	55.8
Methyl tri-*O*-acetyl-2-deoxy-α-*arabino*-hexopyranoside	$CDCl_3$	55.5
Methyl tri-*O*-acetyl-2-deoxy-β-*arabino*-hexopyranoside	$CDCl_3$	57.0
Methyl tetra-*O*-acetyl α-D-mannoside	$CDCl_3$	55.3
Methyl tetra-*O*-acetyl β-D-mannoside	$CDCl_3$	57.3
Methyl tetra-*O*-acetyl-α-D-glucopyranoside	$CDCl_3$	55.8
Methyl tetra-*O*-acetyl-β-D-glucopyranoside	$CDCl_3$	56.7

* The chemical shifts were measured from the methyl carbon absorbance of internal TMS.

Other chemical shift data are available in Tables 6 and 7. It is evident that the chemical shifts of the two ethers are quite similar to each other and to the values found for the α-glycosides. However, when the chemical shifts of the β-glycosides are examined, it is seen that a significant deshielding effect is observed, relative to the other compounds. Such deshielding occurs apparently as a result of electron delocalization from the methyl group and thus accords with the suggestion of Havinga (12) that electron delocalization is an important factor in the anomeric effect. The shorter β-glycosidic bonds reported (21) from X-ray crystallography are also in accord with electron delocalization from the aglyconic bond into the glycosidic bond. It seems assured, therefore, that the α and β glycosidic structures have significantly different electronic structures and, for this reason, adopt slightly different torsion angles as proposed here.

E. The Effect of Substitution at the Aglyconic Carbon of D-Glucopyranosides Upon Their Conformational Properties

1. Vicinal Carbon-Proton Coupling

Because nearly all important glycosides have aglyconic carbons which are more hindered than the methyl group and because of the success of the investigations discussed above, it was decided to examine the conformational properties of glucosides containing bulkier aglycon moieties.

To this end, α- and β-glucosides in which the carbon-13 enriched aglyconic carbon had one, two, or three methyl substituents, were chosen for study. The non-polar nature of the methyl substituent is expected to provide progressive, and straightforward increases in the steric size of the aglycon without the added complication of new hydrogen-bonding or stereo-electronic effects.

The coupling constants of the vicinal aglyconic carbon to anomeric proton system were determined for the α- and β-anomers of the ethyl-1'-^{13}C, and isopropyl-2'-^{13}C and for the β-anomer of *t*-butyl-2'-^{13}C D-glucopyranosides. The results obtained for the free glucosides dissolved in water are presented in Table 11.

It is seen that in this solvent, except for the case of the t-butyl-^{13}C glucoside, (45), increases in the steric bulk of the aglycon do not result in observable changes in the

TABLE 11

Vicinal Aglyconic Carbon to Anomeric Proton Nuclear Spin Coupling Constants of Some Alkyl D-Glucopyranosides in Water

Compound	$^3J_{C,H-1}$ (Hz)
Methyl-^{13}C α-D-	3.8
Ethyl-1'-^{13}C α-D-	3.8
Isopropyl-2'-^{13}C α-D	3.8
Methyl-^{13}C β-C-	4.6
Ethyl-1'-^{13}C β-D-	4.6
Isopropyl-2'-^{13}C β-D-	4.6
t-Butyl-2'-^{13}C β-D	4.2

coupling constants. The decrease observed for the *t*-butyl glucoside may be readily explained by reference to the θ/θ' effect proposed by Karabatsos (38). Deformation of the glycosidic bond angle C_1-O_1-C_A is known to occur and, for example, the bond angle found by X-ray crystallography (113) in the sucrose moiety of planteose is 122°, which may be compared to the more normal range of 111° to 116° (78). The sucrose structure has the highly hindered tertiary carbon-2 of fructose as one portion of the glycosidic linkage. Thus, it has a tri-substituted aglyconic carbon similar in steric bulk to the *t*-butyl group. It seems reasonable, therefore, to assume that bond angle deformation, similar to that found in planteose, will occur in the *t*-butyl glucoside with consequent reduction in coupling constant.

From these results, following arguments similar to those discussed for the methyl glycopyranosides, it is concluded that the α-a and β-a rotamers predominate in the free glucosides examined in this section. Furthermore, the increases in steric bulk of the aglycon do not result in changes in the torsion angle between aglyconic carbon and anomeric proton for the aglycon series methyl, ethyl and isopropyl.

When the per-*O*-acetylated glucosides were examined using chloroform as solvent, results were obtained which contrasted sharply with the above. This can be seen from Table 12.

For these compounds in chloroform a significant increase in the observed coupling occurs in both anomeric series. The suggestion is obvious that the increased steric bulk of the aglycon, interacting with the ring oxygen, causes a decrease in the torsion angle between the coupled nuclei. As has been noted several times, only slight decreases in this angle may result in large increases in coupling constant. When the values of the coupling constants for methyl-^{13}C and isopropyl-2'-^{13}C α-D-glucoside are compared with the angles associated with them on the Karplus-type

TABLE 12

Vicinal Aglyconic Carbon to Anomeric Proton Nuclear Spin Coupling in Some Alkyl Tetra-*O*-acetyl-D-glucopyranosides in Chloroform-*d*

Compound	$^3J_{C,H-1}$ (Hz)
Methyl-^{13}C α	3.6
Ethyl-1'-^{13}C α	3.9
Isopropyl-2'-^{13}C α	4.2
Methyl-^{13}C β	4.3
Ethyl-1'-^{13}C β	4.4
Isopropyl-2'-^{13}C β	4.7
t-butyl-2'-^{13}C β	4.0

curve (Fig. 17) it is seen that a torsion angle of 53° corresponds to the coupling observed in the methyl compound, while an angle of 50° would correspond to the coupling constant of the isopropyl compound. Similarly, in the β-anomers, a torsion angle of 49° corresponds to the observed coupling in the methyl glucoside and, for the isopropyl compound, a torsion angle of 46° is obtained. These angles are well within the range of values which might reasonably be expected. Thus, it is concluded that identical increases in the steric bulk of the aglycon cause identical conformational responses in these compounds, and also, that the relatively smaller torsion angle of β-glycosides is retained.

The invariance in torsion angle found for the free glucosides in water seems to result from stabilization of the particular torsion angle rather than from reduction in the non-bonded interactions involved and could arise from a specific solvation effect by water. This suggestion is indicated by the observation of decreased coupling in both *t*-butyl compounds, which infers the presence of strong interactions causing θ/θ' bond angle deformation (see page 23).

2. Rotation About the Aglyconic Bond

The lack of variation observed in the vicinal coupling, between the aglyconic carbon and the anomeric proton, of the methyl-^{13}C, ethyl-1'-^{13}C, and isopropyl-2'-^{13}C D-glucopyranosides in water, may be contrasted with the steady increase in the magnitude of the contribution of the aglycon moieties to the molecular rotation constants of their glycosides. Table 13 presents the observed $[M]_D$, values for a series of D-glucosides and their parent α- and β-D-glucopyranoses.

TABLE 13

A Comparison of The Molecular Rotation Constants, in Water, of Some Simple Alkyl Glucopyranosides and Their Parent Glucopyranose Anomer

Compound	$[M]_D$	$\Delta\ [M]_D$*	Reference
α-D-Glucose	+202°	—	74
Methyl α-D-glucoside	+309	+107°	74
Ethyl α-D-glucoside	+314	+112	102
Isopropyl α-D-glucoside	+324	+122	104
Cyclohexyl α-D-glucoside	+349	+147	114
β-D-Glucose	+ 34	—	74
Methyl β-D-glucoside	- 66	-100	74
Ethyl β-D-glucoside	- 76	-110	102
Isopropyl β-D-glucoside	- 84	-118	104
Cyclohexyl β-D-glucoside	-104	-138	114

* $\Delta\ [M]_D = [M]_D$ alkyl glucoside - $[M]_D$ glucose.

Since it has been established above that these compounds exist virtually exclusively in the α-a and β-a rotamers, it appears that the observed increases in $\Delta\ [M]_D$ must arise from optically active three-bond units of asymmetry involving the aglycon. The contribution of the isopropyl and cyclohexyl groups is especially large and these have recently been analysed successfully (114), in terms of their relative rotamer populations about the aglyconic bond.

From these interpretations of coupling constant and optical rotation data, it is concluded that the principle mode of accommodation of the increases in non-bonded interaction energies, in aqueous medium, is rotation about the aglyconic bond. The presence of these interaction energies leads to preferred conformations about the aglyconic rather than the glycosidic bond.

F. Implications of This Research

A difficulty, which has existed for some time in the analysis of the complex biological glycosides of medical and industrial interest has been the problem of the detection of the conformational properties of the glycosidic and aglyconic bonds. Much information from X-ray crystallography (78) has been available concerning the crystalline state, however, the presence of steric forces peculiar to that state has made the applicability of X-ray data to solvated molecules problematical. With the development of a satisfactory Karplus-type equation for vicinal carbon to proton coupling, a method was made available for the detection of conformational properties in solution. With this method it has been shown that the smaller torsion angles between aglyconic carbon and anomeric proton of β-glycosides, which are generally found in the crystalline state, are also found in solution. It should be noted, however, that the rather small range of coupling constants found for β-glycosides in this work is indicative that only a small range of torsion angles exists in solution. It may be concluded therefore, that the wide range of torsion angles which have been reported for various crystalline β-glycosides are the result of specific crystal field forces. The α-glycosides also seem to have a small range of torsion angles and the indication is, therefore, that in the solvated state there exists a certain levelling effect which causes roughly equal torsion angles to be adopted by compounds of the same anomeric configuration. This tendency was especially notable in water, suggesting that X-ray data for crystalline cellobiose (ϕ = 43.8°) (33), and methyl β-D-cellobioside (ϕ =31.1°) (115), may not be valid in solutions of these molecules. In turn, the helicity of β-oligosaccharides in water may differ significantly from expectations based on X-ray data.

It has been shown here, for a series of alkyl aglycons and glycopyranoid structures, that only the α-a and β-a rotamers of the glycosidic bond have important populations. As a result of this property of the glycosides, it has become possible, utilizing empirical rules for optical rotation, to analyse the rotamers of the aglyconic bond.

Thus, in combination, these two techniques have been used (114) to define the entire rotameric structure of certain glycosides, and therefore, their complete secondary structure. The insights obtained in such investigations will no doubt prove invaluable in the solution chemistry of more complex carbohydrate molecules. An example is provided by the conclusion (114) that in aqueous solution, only weak hydrogen-bonding is present between the 2'-hydroxyl and pyranoid ring oxygen atom in (1'R)-*trans*-2'-hydroxylcyclohexyl β-D-glucopyranoside. This compound is structurally similar to cellobiose in this regard, and, therefore, these results may indicate that such hydrogen bonds are weak in solvated cellobiose as well, and do not play a role in determining its secondary structure.

A further implication of this research is that the values assigned to the free energies of non-bonded interactions in molecules of fixed conformation may require modifications for applications in the conformational analysis of the energies of rotamers about polar bonds involving heteroatoms. Recently, suggestions as to the attractive forces between nuclei and electrons have been put forward (27). Similarly, it has been calculated (116) that properly oriented lone electron pairs may interact in an attractive sense by forming bonds with the orbitals of neighbouring atoms. By the application of vicinal carbon to proton coupling experiments, previously unknown non-bonded and "bonded" interaction energies of this type may become established.

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